Bulk Transport: Exocytosis & Endocytosis

Lecture 12

Objectives

Understand/know/focus on/note

- what a vesicle is and how it has multiple purposes
- the mechanisms and purposes of
  - endocytosis
  - exocytosis
  - phagocytosis
  - pinocytosis
  - receptor-mediated endocytosis
- examples of how these processes occur in human cells

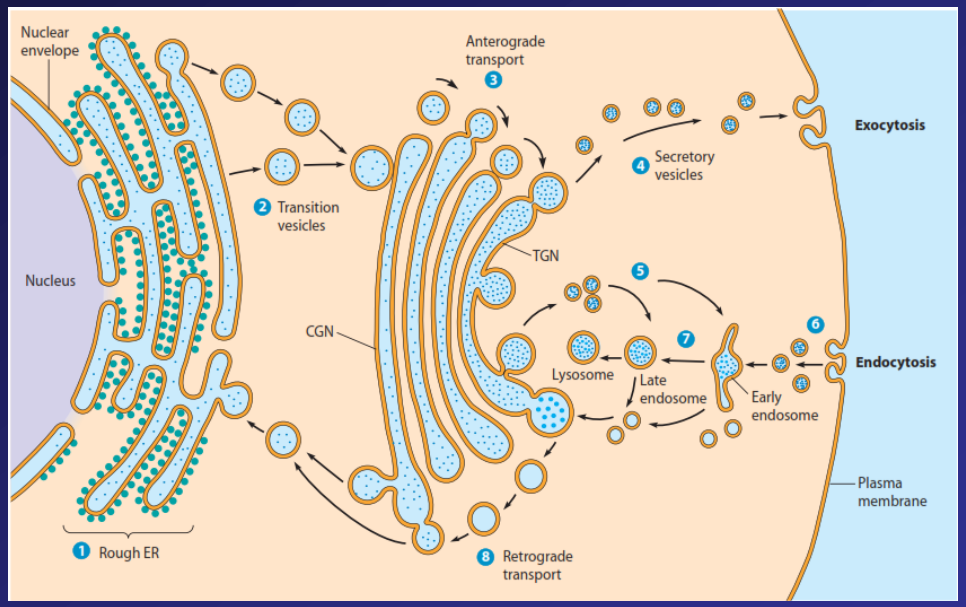
Vesicles in Cells

Vesicles are membranous containers

- The membrane is the same phospholipid bilayer that makes up all membranes
- The membrane proteins may differ from those on the cell surface (the plasma membrane), unless the vesicle is being prepared for becoming part of the plasma membrane
- The inner contents of a vesicle can vary greatly, depending on the fated function of the vesicle

Vesicle Formation & Fusion

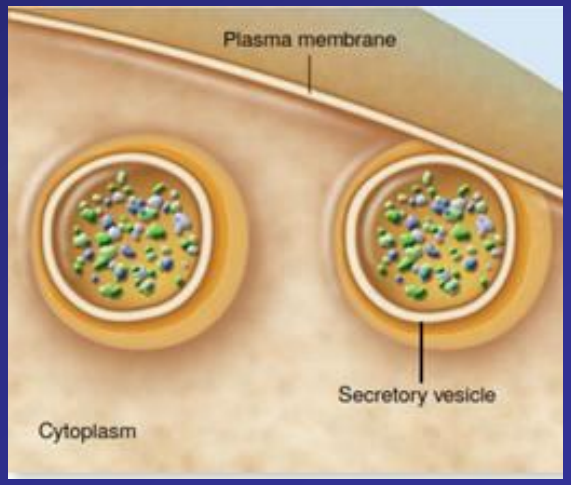
- The proteins and phospholipids that make up the plasma membrane start forming in the endoplasmic reticulum (ER), a network of flattened membranous sacs. The ER may be associated with ribosomes, where protein synthesis is occurring
- Vesicles form off the ER and move to another series of flattened membranous sacs called the Golgi complex, where particularly glycoproteins will get modified with their oligosaccharides
- Some vesicles will eventually make their way to the surface of the plasma membrane and fuse with it



Exocytosis 1 of 3

- Exocytosis refers to any process in which the cell forms a system for expelling or secreting contents from within its cellular container
- In exocytosis, the inner space of the vesicle will fill with a substance that is to be secreted from the cell

  For example, neurons produce vesicles containing neurotransmitters such as acetylcholine or epinephrine
- As the vesicle matures with the filling of its space, it approaches the surface of the plasma membrane and makes contact with it

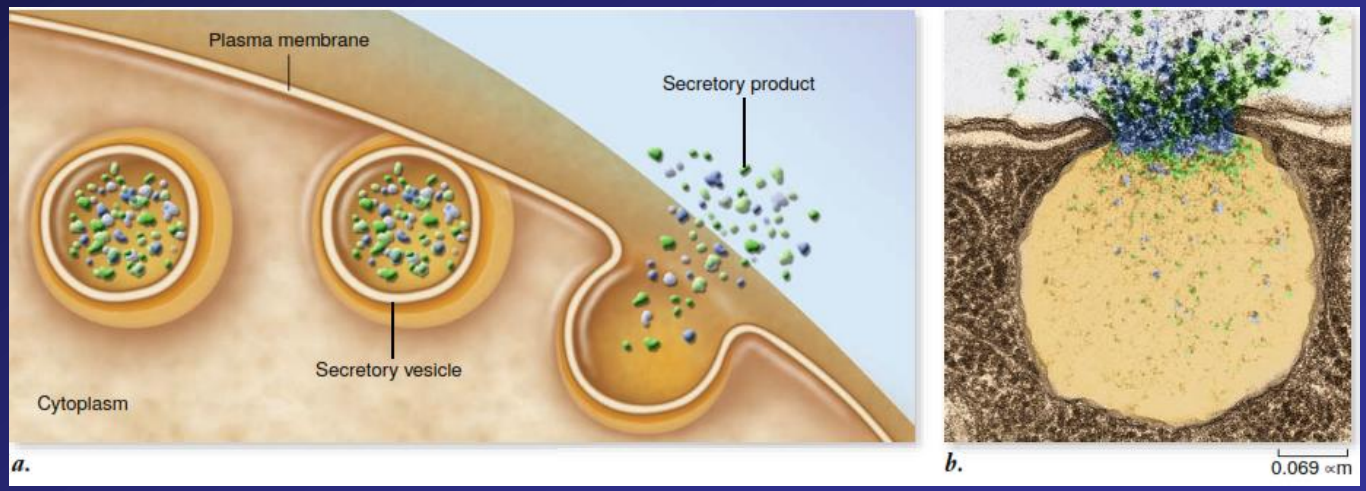


Exocytosis 2 of 3

- A signal will then be transmitted to the plasma membrane

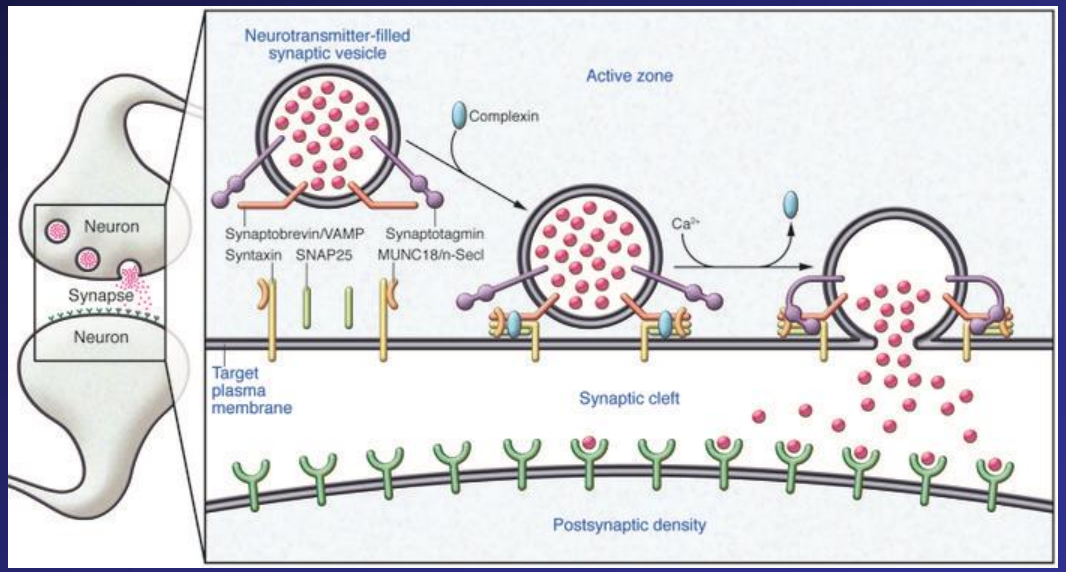
  for neurons, it can be an action potential
- The signal will then cause Ca^{2+} channels on the plasma membrane to open
- with Ca^{2+} levels higher outside of a cell then inside, Ca^{2+} rushes in

  In neurons, the Ca^{2+} channels can be triggered by membrane voltage: voltage-gated Ca^{2+} channels



Exocytosis 3 of 3

- The increased Ca^{2+} then starts a chain of events that make the membranes of the vesicle fuse with the plasma membrane, and the chemical contents of the vesicle are released to the outside of the cell



The figure at left shows a lot of the molecular details of Ca^{2+}-mediated vesicle fusion in a neuron that are known but beyond the scope of this course

Endocytosis

- Endocytosis is about the cell using a process in which the cell membrane invaginates, enclosing something from the extracellular medium within the invagination, and then fusing the plasma membrane to form a vesicle

3 Types

1. phagocytosis
2. pinocytosis
3. receptor-mediated endocytosis

   also referred to as adsorptive pinocytosis

Phagocytosis

- Cells "eating" large particles
- Cell engulfs large or solid material (> 0.5 μm)

  clump of bacteria, cell debris, inanimate particles (e.g. asbestos fibers or glass)
- Lower animals use this process for their nutrition

  e.g. protozoa, sponges, flatworms
- Phagocytic cells in humans are neutrophils and macrophages

  not used for nutrition, but rather body defense against bacteria, foreign substances, and eliminating debris from dead cells

Phagocytosis Overview

- Phagocytes (cells capable of phagocytsis) move by an ameboid motion in which cytoplasm flows into cell membranes to cause membranes to envelop target
- This happens in response to the target activating cell receptors
- The cytoplasmic extensions are called pseudopods
  pseudo: sham, mock, deceptive, ersatz; *podia*: feet
- A vesicle forms around the target called a phagosome
- The phagosome fuses with a lysosome to form a phagolysosome
- The phagolysosome vesicle activates H^+-transporting membrane proteins that pump H^+ from the cytosol into the vesicle interior, greatly acidifying it

Phagocytosis Mechanism 1 of 2

1. Cell membrane folds around particle to form food cup
2. Membrane invaginates and folds extend further around the particle

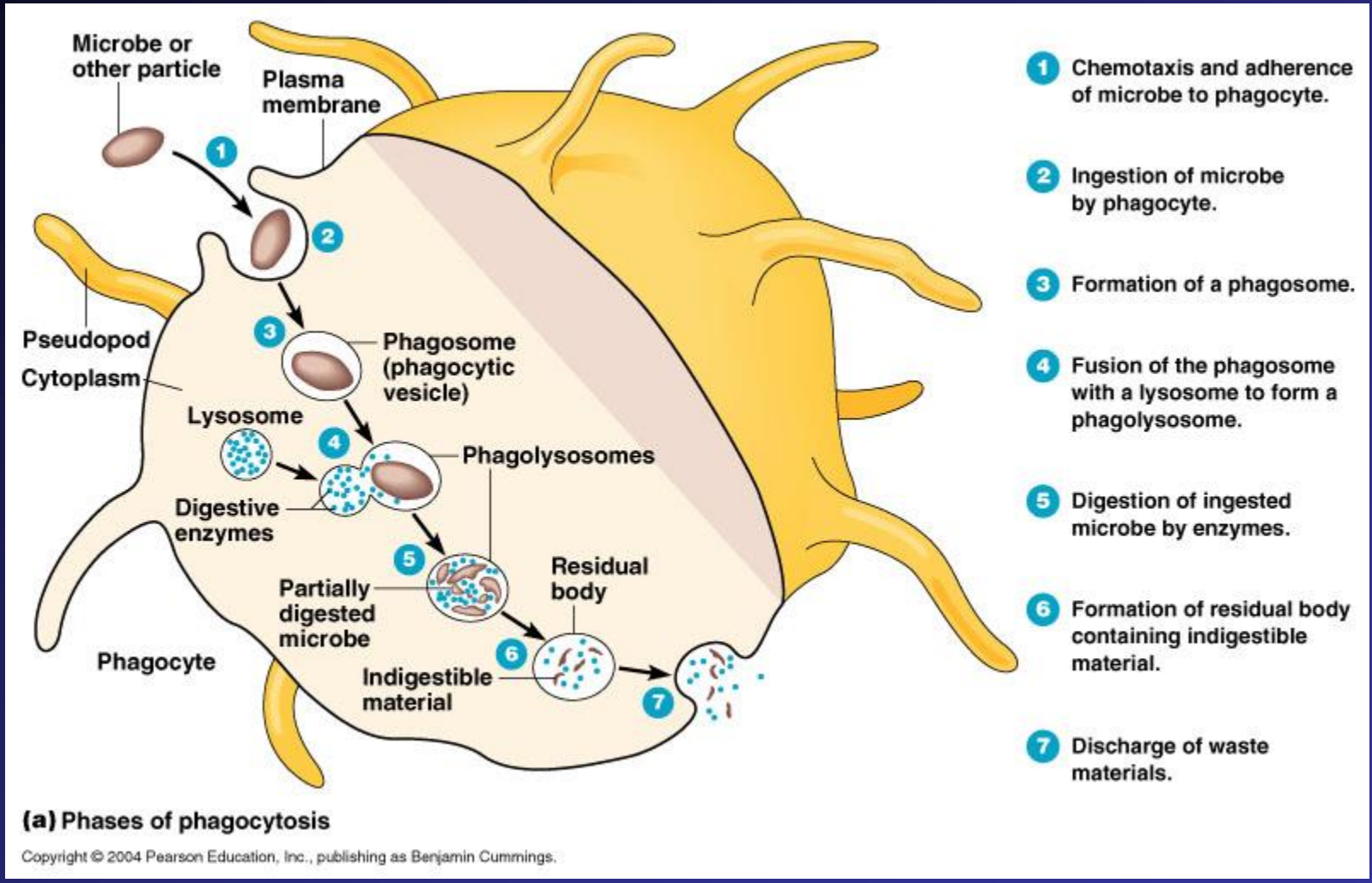
   Eventually the particle is completely engulfed
3. Membrane pinches off to form a free phagocytic vacuole in cytoplasm
4. Phagocytic vacuole moves towards Golgi region
5. Phagocytic vacuole merges with a primary lysosome: this forms a secondary heterophagic lysosome

Phagocytosis Mechanism 2 of 2

6. The internal pH of lysosome is reduced by proton pumps in lysosomal membrane

   At low pH (< pH 5) lysosomal enzymes are activated, and these digestive enzymes break down the particle into smaller molecules (destroying biological activity)

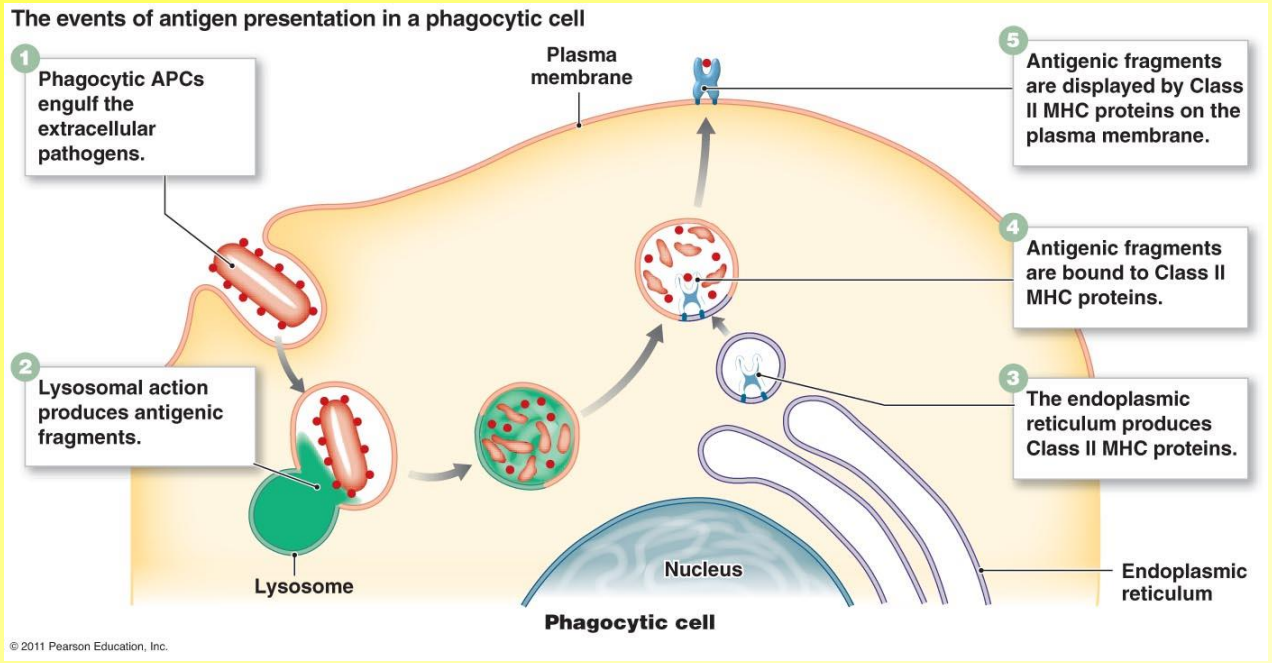
7. Undigestible waste may have any of these fates:
   - In lower organisms, it is excreted by exocytosis
   - In mammalian phagocytes, some stay in the cell and contributes to cell aging
   - Neutrophils may "eat until they burst" because they don't release old waste



(a) Phases of phagocytosis

Macrophages Have Additional Function

- Macrophages not only break down foreign material
- Oligopeptide (short polypeptide) fragments (from 9-11 amino acids long) are allowed to bind to major histocompatibility (MHC) class II molecules and presented on the macrophage cell surface
- If a T lymphocyte recognizes the MHC II-oligopeptide antigen complex, it gets activated, because the MHC II-oligopeptide antigen complex is foreign! This causes an immune reaction that builds up antibodies and also cytotoxic cells that recognize the complex



- Self antigens don't activate T lymphocytes because all T lymphocytes that could recognize them were exterminated a long time ago during the development of the immune system

Pinocytosis

pino-: drinking; -*cyt*-: cell; -*osis*: condition of

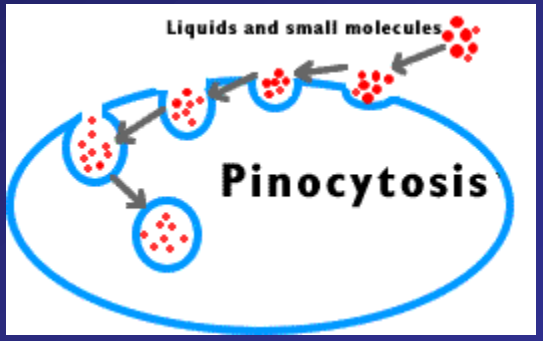
also called fluid-phase endocytosis

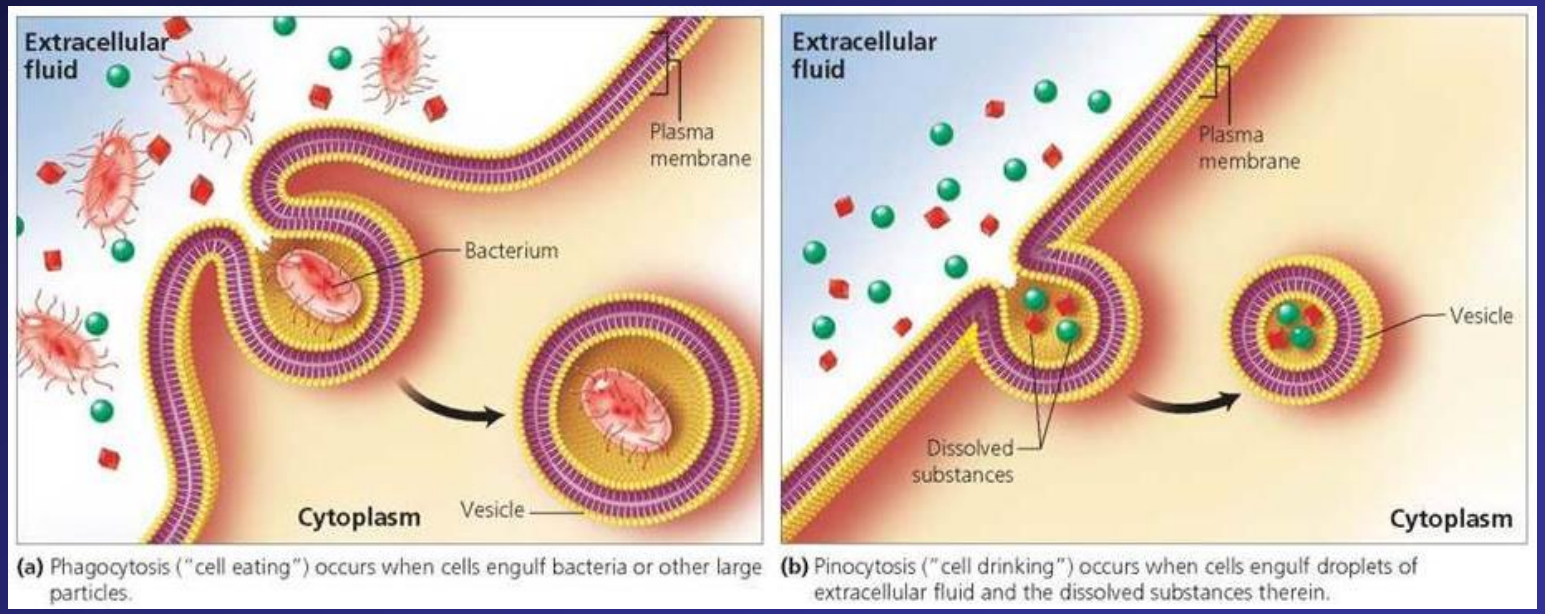
- Infolding plasma membrane surrounds very small volume of extracellular fluid containing dissolved molecules ("droplet"): droplet enters the cell
- Pinocytosis is a routine activity of most cells, because they sample extracellular fluid in nonselective way
- Intestinal epithelial cells use pinocytosis a great deal in order to absorb nutrients
- Pinocytic vesicles are returned to surface after contents absorbed intracellular to restore membrane phospholipids

Pinocytosis Mechanism

1. Macromolecules bind to general receptors on plasma membrane
2. Membrane invaginates, drawing particles into a "cupped" region
    - Different from a phagocytic "food cup" because there are no externally projecting folds
3. Cupped region becomes completely enclosed by invagination
4. Pinches off to form a free pinocytic vesicle within the cytoplasm

- illustration below shows difference between phagocytosis & pinocytosis





(a) Phagocytosis ("cell eating") occurs when cells engulf bacteria or other large particles.

(b) Pinocytosis ("cell drinking") occurs when cells engulf droplets of extracellular fluid and the dissolved substances therein.

Receptor-Mediated Endocytosis

- Also called adsorptive pinocytosis
- Allows cells to concentrate material present only in small amounts in extracellular fluid
- Uses specialized membrane receptors (as seen in cell signaling) that target molecules outside cell
- Process leads to membrane infolding to form vesicle with many accumulated target ligand molecules brought into the vesicle
- On inside of cell, a scaffold of proteins of different kinds helps to form a vesicle: the scaffolding plus the pit are called coated vesicles or coated pits
- NOTE: the receptors are NOT the scaffolding proteins!!

Receptor-Mediated Endocytosis Features

- Identified scaffolding proteins
  - clathrin
  - caveolin (supposedly not widely accepted: status?)

Examples of receptor-mediated endocytosis

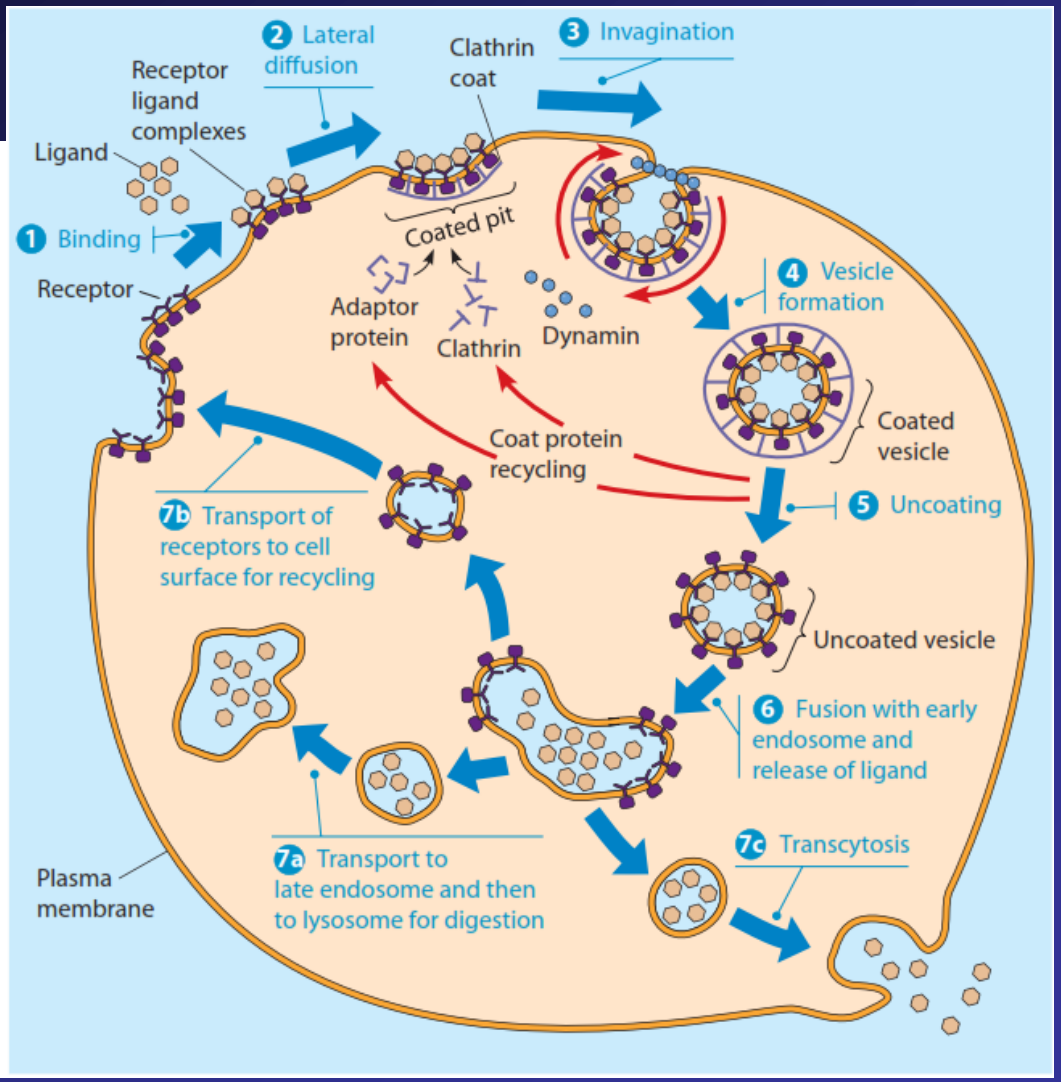
- Receptors for low-density lipoprotein (LDL) collect & take in LDL particles, largely to obtain cholesterol
- The iron (Fe^{3+})-carrying plasma protein transferrin (TRF) is taken in by the RME process for cells with TRF receptors
- Pathogenic bacteria have adapted to make use of RME process in order to introduce their toxins into the cell: e.g. diphtheria toxin

Receptor-Mediated Endocytosis Mechanism

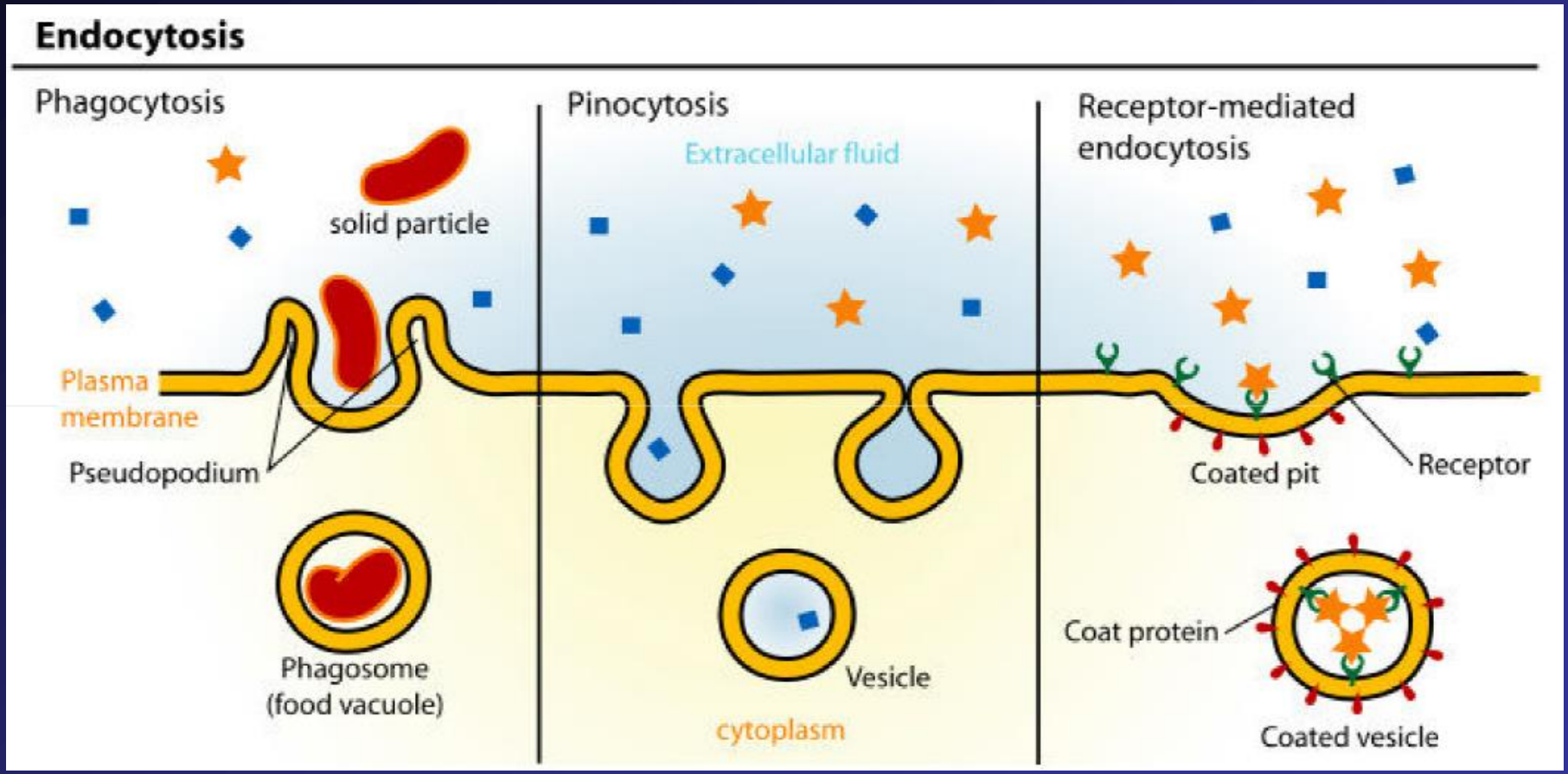
1. Ligand binds to specific receptors on the cell membrane, triggering internalization process
2. Ligand-receptor complexes move laterally within membrane to collect into forming coated vesicles
3. Endocytosis occurs in region of coated vesicle
4. Coated vesicle forms in cytoplasm
5. Coated vesicle may fuse with other vesicles which are not coated
6. The clathrin coat dis-assembles and proteins recycled back plasma membrane
7. Vesicle membrane has ATP-dependent proton pump that acidifies the vesicle: acidification causes ligand separation from receptors
8. Ligands are processed further to obtain substance (cholesterol, iron) for cell utilization. Receptors are recycled back to plasma membrane.

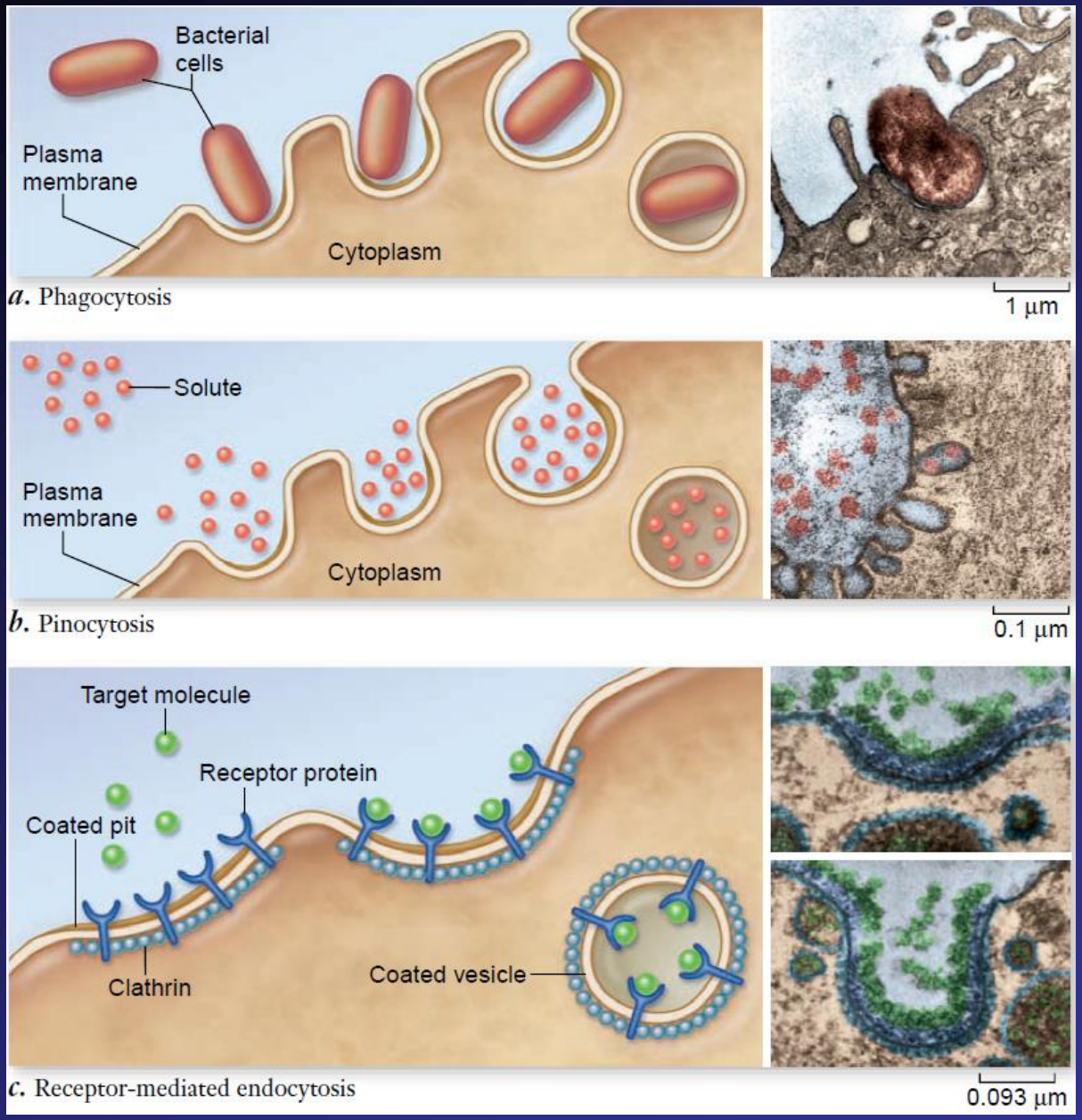
- Becker, p 345

FIGURE 12-15 Receptor-Mediated Endocytosis. During receptor-mediated endocytosis, ❶ the molecules that will be internalized bind to specific receptors on the surface of the plasma membrane. ❷ Receptor-ligand complexes accumulate in coated pits, where ❸ invagination is facilitated by adaptor protein, clathrin, and dynamin on the cytosolic surface of the membrane. The result is ❹ an internalized coated vesicle that ❺ quickly loses its clathrin coat. The uncoated vesicle is now free to ❻ fuse with other intracellular membranes, usually a membrane surrounding an early endosome, where internalized material is sorted. The fate of the receptors and the ingested molecules depends on the nature of the material. Transport vesicles often ❼a carry material to a late endosome for digestion. Alternative pathways include ❼b recycling to the plasma membrane or ❼c transport to another region of the plasma membrane and exocytosis (called transcytosis). For clarity, the nucleus is not shown.



Bulk Transport Visual Summary





General review of endocytosis from Raven *Biology*

Reading (Sources)

- Becker's WotC: pp 341-355
- Raven: Chap 5.6
- Marieb: pp 79-81

Crossing the Membrane Comparison

Property	Passive Diffusion	Facilitated Diffusion	Active Transport	Co-Transport
specific membrane protein required	no	yes	yes	yes
solute transported against gradient	no	no	yes	yes
coupled to ATP hydrolysis	no	no	yes	no
coupled to movement of cotransported molecule/ion down its gradient	no	no	no	yes
examples of molecules transported	O_2, CO_2, steroid hormones	glucose, amino acids, ions, water	ions, lipids, small hydrophilic molecues	glucose, amino acids, various ions sucrose

adapted from Lodish et al., eds., *Molecular Cell Biology*, 5th Ed

This table compares the various ways solutes move across the cell membrane