Cell Organelles:

Endoplasmic Reticulum, Golgi Complex, Lysosomes, Peroxisomes

Lecture 15

Objectives

- Describe the structure, types and functions of endoplasmic reticulum (ER)
- Describe the role of ER in detoxification process and processing of proteins
- Compare between rough and smooth ER
- Describe the structure and function of Golgi complex
- Explain how Golgi complex acts as a “Traffic Director” of cellular proteins
- Describe the structure, activation process, and function of lysosomes
- Describe the function and origin of peroxisomes

Endoplasmic Reticulum (ER)

- *endoplasmic* "within the cytoplasm"; reticulum "a little net"
- continuous network of flattened sacs, tubules, and associated vesicles enclosed by closely apposed (parallel) membranes
- the sacs are called cisternae (cisterns)
- the space within called the lumen
- the ER is continuous with the outer nuclear membrane
- the area of the ER membrane is 50-90% of all the membranes of the cell

Two types of ER

1. rough ER
2. smooth ER

Rough ER

- Cytosolic (not lumenal) side of the ER membrane is studded with ribosomes: origin of "rough" ER naming
- Ribosomes synthesize polypeptides (proteins) & can dock to ER membrane when the protein is fated to be exported from cell or embedded in membrane
- Nascent polypeptides thread into ER lumen during synthesis; later they vesiculate from ER to travel to Golgi before heading to cell surface

Rough ER (RER) Facts

- RER ribosomes make all proteins fated to be secreted or embedded in a membranous part of cell: plasma membrane, lysosome
- RER is well-developed/abundant in cells with functions to make secretory proteins
  - antibody-producing plasma cells
  - hepatocytes making plasma proteins
- The cell's plasma membrane components have origin in ER: all integral proteins and phosphoplipids arise from it
- Lipid synthesis enzymes for making membrane lipids are present on cytosolic side of ER membranes

Smooth ER (SER)

- When present in cells, smooth ER is continuous with rough ER

Cell types with extensive SER and why:

- Cells making steroid hormones
  cells in ovary & testes & adrenal cortex make steroids from cholesterol, which is a very lipophilic molecule

  the SER provides chemical environment for the kind of enzymes requiring a water-free microenvironment to perform these reactions
- Liver cells (hepatocytes) storing glycogen
  the liver regulates blood glucose levels by breaking down glycogen to glucose and releasing glucose to blood

the SER features the enzyme glucose-6-phosphatase, which catalyzes the reaction below so that glucose can exit the cell (remember that the enzyme hexokinase phosphorylates glucose to traps it into cells after entry)

glucose-6-phosphate + H_2O → glucose + P_i (P_i = $H_2PO_4^-$)

Smooth ER (SER)

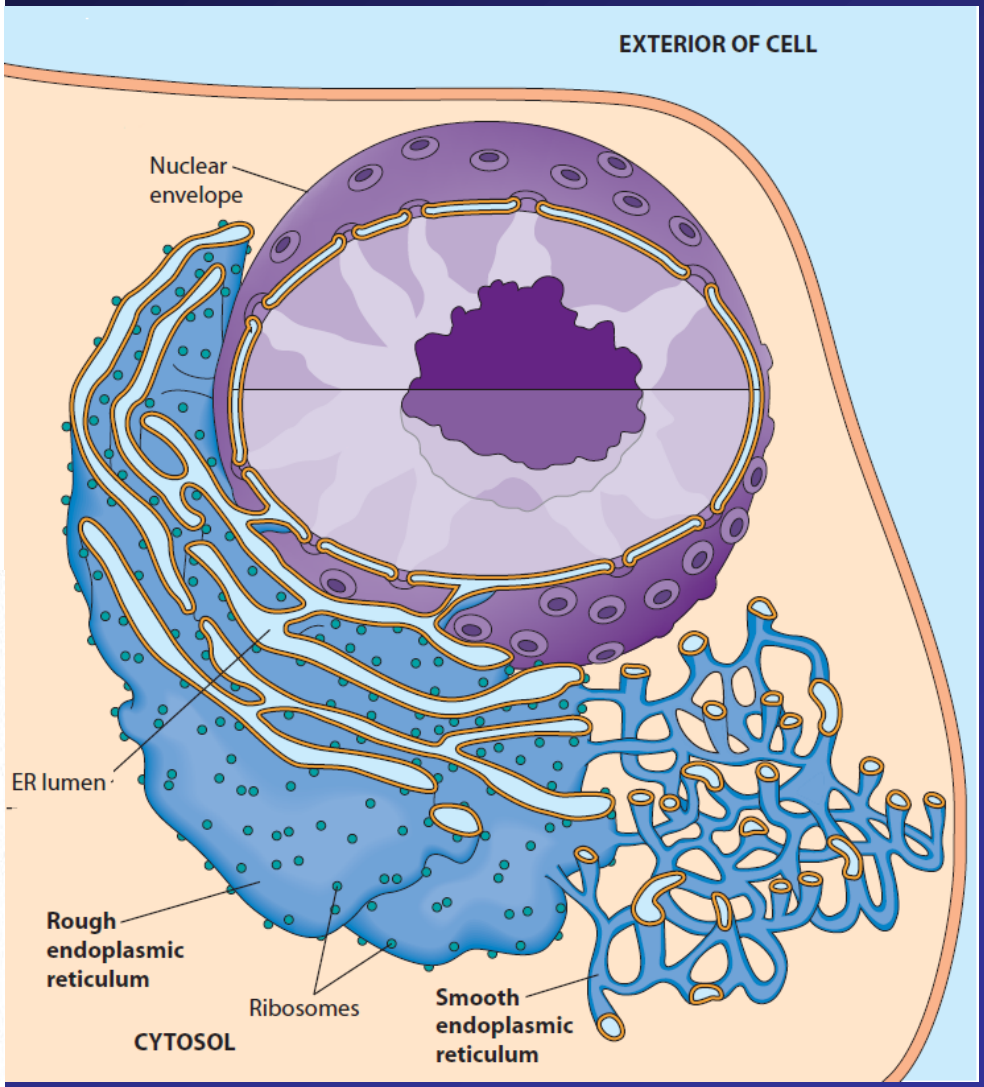
- Muscle cells using calcium (Ca^{2+})

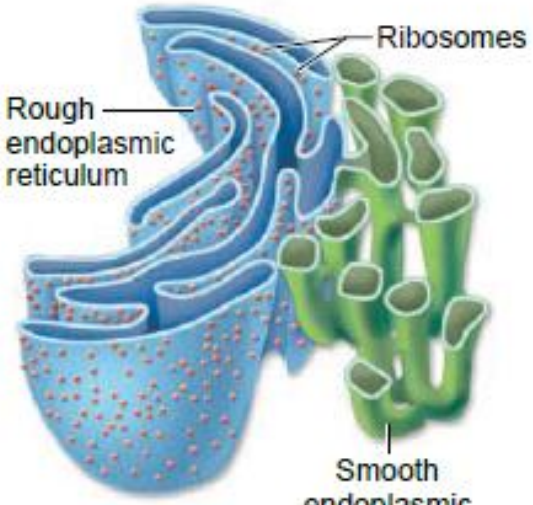
  The sarcoplasmic reticulum (SR) is a special form of the ER in muscle cells largely used as a store of Ca^{2+} ion

  Ca^{2+} is required as part of the interaction of the proteins myosin and actin in muscle contraction, and it must be removed quickly when muscle is to be relaxed
  The SR membrane has many Ca^{2+} ATPase pumps to pump Ca^{2+} out of the cytosol and into the lumen of the SR

  Note: when contraction is desired, the Ca^{2+} ions—at a high concentration in the SR lumen—are released into the cytosol of muscle cells by voltage changes by depolarization in the SR membranes, which open voltage-dependent Ca^{2+} transmembrane channel proteins to allow Ca^{2+} to rush into the cytosol


(a) Rough endoplasmic reticulum
0.25 μm
(b) Smooth endoplasmic reticulum
0.25 μm


EXTERIOR OF CELL
Nuclear envelope
ER lumen
Rough endoplasmic reticulum
Ribosomes
CYTOSOL
Smooth endoplasmic reticulum



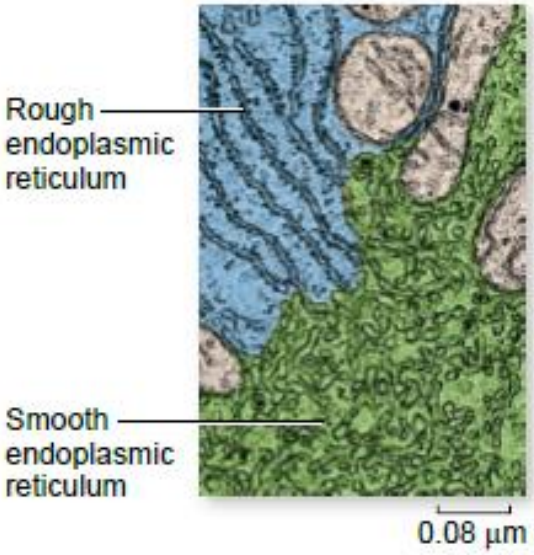


Figure 4.10 The endoplasmic reticulum. Rough ER (RER), blue in the drawing, is composed more of flattened sacs and forms a compartment throughout the cytoplasm. Ribosomes associated with the cytoplasmic face of the RER extrude newly made proteins into the interior, or lumen. The smooth ER (SER), green in the drawing, is a more tubelike structure connected to the RER. The micrograph has been colored to match the drawing.

Smooth ER In Detoxification

- The SER provides the proper chemical/catalytic environment for a group of enzymes known as cytochrome P450 mono-oxygenases
- Many toxic compounds that enter the body through inhalation by lungs, ingestion through intestine, and by dermal absorption are highly lipophilic (nonpolar, hydrophobic)
- The P450 enzymes get these toxicants (which can also be drugs) into their active site, and they create the equivalent of a chemical bomb to alter their unreactive chemical structure
- This "chemical bomb" is a reactive oxygen species (ROS) that reacts with everything, but the enzyme tries to create the reaction so that the ROS hits the toxic chemical first
- Sometimes the P450 enzyme is itself destroyed by the ROS product it forms in the active site & sometimes the ROS gets away without reacting, requiring the ROS to be taken out by other enzymes (see peroxisome slide explanation)

SER: Biotransformation 1 of 3

- The Cytochrome P450 (CYP) reaction is:
  RH + NADH/NADPH + H^+ + O_2 → ROH + NAD/NADP+ + H_2O
- The RH (toxicant, drug) is usually a very hydrophobic (lipophilic) molecule
- Cellular biochemistry prefers an aqueous environment: it doesn't prefer hydrophobic molecules, so the P450 systems tries to make lipophilic substances like toxicants and drugs polar with its reactions
- To make polar what is nonpolar typically involves oxygenating a molecule
  - recall that O and N atoms create polarity in covalent bonds as they are the most electronegative of the atoms
  - the product is hydroxylated (a relatively lipophilic C–H bond will be converted to a polar covalent C–OH bond)

SER: Biotransformation 2 of 3

- All cells in the body have some ability to deal with toxic substances that enter them
- Hepatocytes in the liver have a huge array of enzymes in the SER that detoxify these substances
- Phase I Biotransformation

  These are enzymes that fall into THREE classes
  - hydrolases: they add water to chemical groups with the purpose of creating –OH groups (increasing polarity & reducing lipophlicity)
  - reductases: they usually react with relatively not-as-polar carbonyl (C=O) groups, like aldehydes & ketones, and try to reduce them to a-bit-more-polar alcohols (–OH groups)
  - oxygenases: this is the class in which P450 enzymes fall, which try to put –OH groups using reactive oxygen species on very lipophlic substances

mono-oxygenase (P450) enzyme reaction

$$RH + (NADH \text{ or } NADPH) + H^+ + O_2 \rightarrow ROH + (NAD^+ \text{ or } NADP^+) + H_2O$$

SER: Biotransformation 3 of 3

- Phase II Biotransformation
  - This process is often called conjugation, because a chemical group is conjugated to the toxicant/drug/poison
  - The Phase II enzymes attach the chemical group usually to an –OH group that was probably put there by Phase I enzymes or which already existed
  - glucuronidation

    a glucuronic acid is put on the –OH group

    glucuronic acid=glucose with number 6 carbon as a —COO– group on it, makes it negatively charged
  - sulfation

    —SO_4^{2-} group put on the –OH group

    makes it negatively charged
  - methylation
  - acetylation

The conjugated group increases the polarity even more (even making it ionic in some cases) and helps to mark or tag the toxicant for excretion by urine or through bile typically

ER-Synthesized Polypeptides

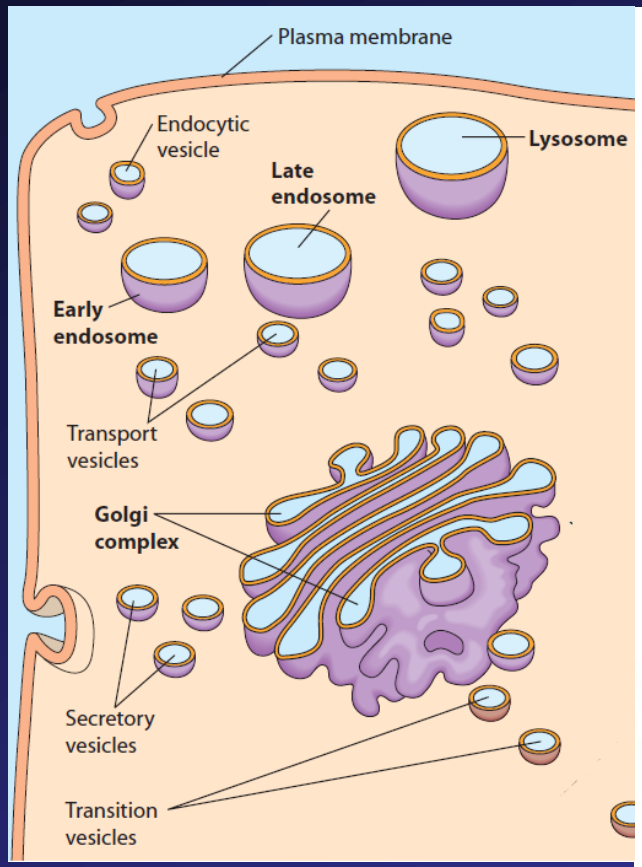
- After synthesis in the rough ER, protein goes into vesicles budding off the ER
- Vesicles are guided through the cytoplasm by microtubules

  microtubules are filamentous proteins
  acting like "cellular cables"

- Vesicles fuse with the *cis* face of the Golgi complex
- Complete proteins go into vesicles membranes or inner space, budding off the *trans* face of the Golgi to their final site depending on protein funciton
- Secretory proteins enter the vesicle space, with vesicles moving to contact plasma membrane

  Exocytosis usually occurs in response to event/signal

- Transition vesicles bud off from ER and fuse to the cis-Golgi network (cis face)
- Within the Golgi, vesicles bud off and fuse to other Golgi sacs in moving from trans to cis face of Golgi
- Enzymes in Golgi modify particularly the oligosaccharide molecules of glycoproteins on the membrane.



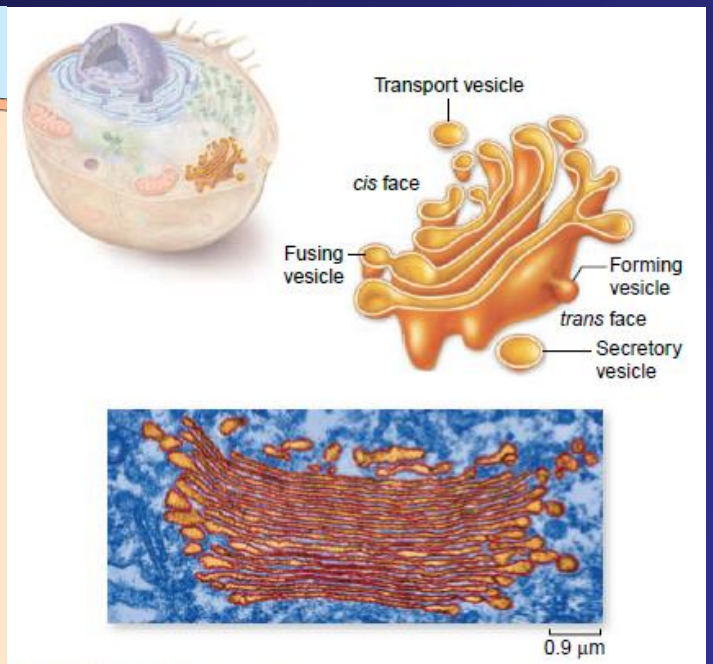
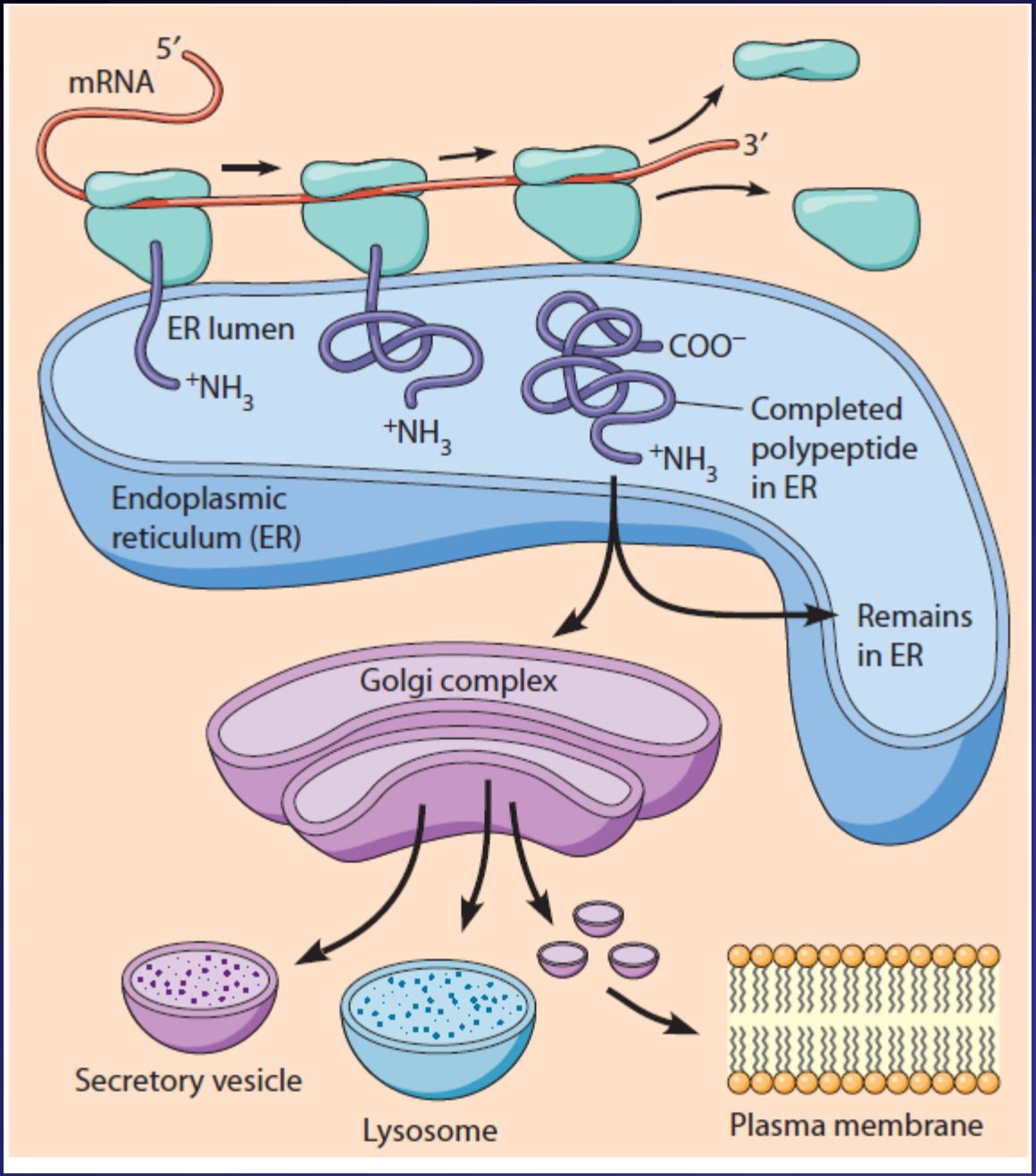
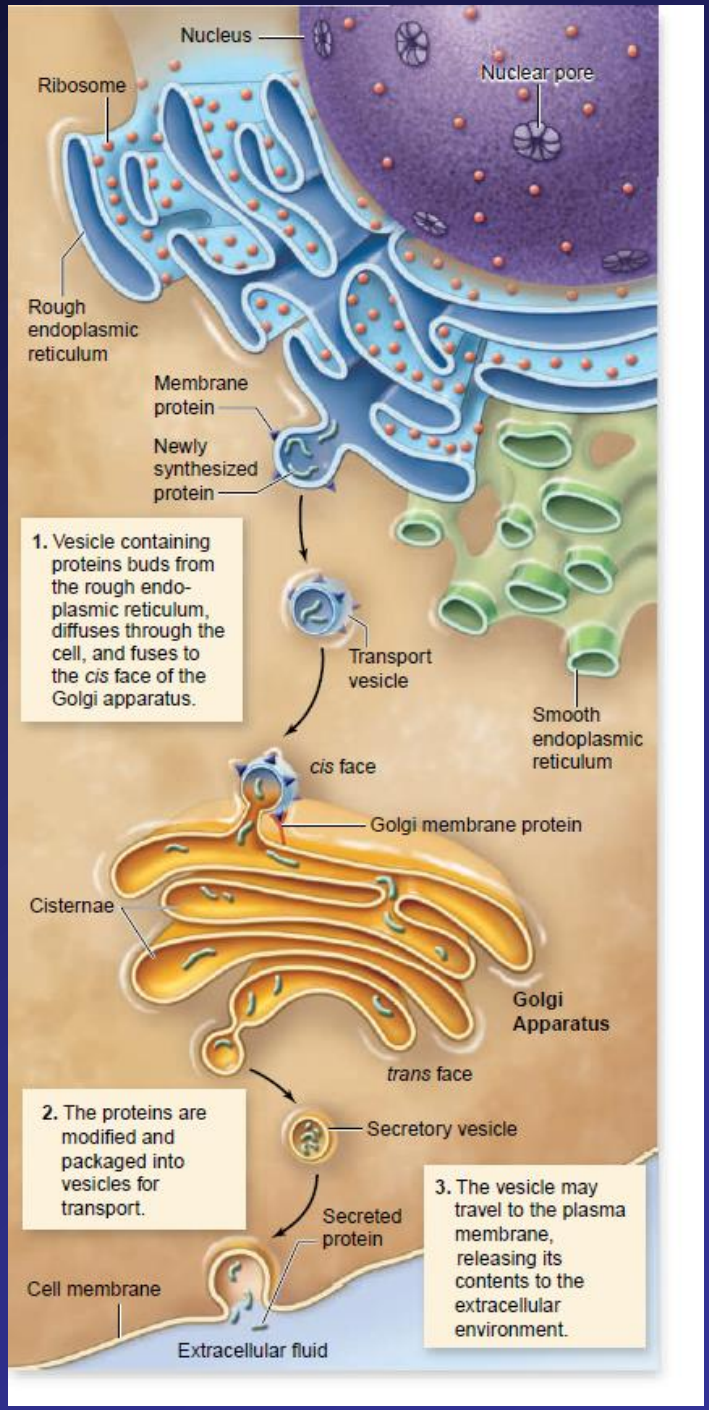


Figure 4.11 The Golgi apparatus. The Golgi apparatus is a smooth, concave, membranous structure. It receives material for processing in transport vesicles on the *cis* face and sends the material packaged in transport or secretory vesicles off the *trans* face. The substance in a vesicle could be for export out of the cell or for distribution to another region within the same cell.



Although we have not covered protein synthesis yet (shown in top of illustration), polypeptides formed in the ER (*not* cytosol) will be in the lumen of ER and will move (by vesicles) from Golgi and ultimately to secretory vesicles, lysosomes, or to the cell surface on the plasma membrane


Nucleus
Nuclear pore
Ribosome
Rough endoplasmic reticulum
Membrane protein
Newly synthesized protein
1. Vesicle containing proteins buds from the rough endoplasmic reticulum, diffuses through the cell, and fuses to the cis face of the Golgi apparatus.
Transport vesicle
Smooth endoplasmic reticulum
cis face
Golgi membrane protein
Cisternae
Golgi Apparatus
trans face
2. The proteins are modified and packaged into vesicles for transport.
Secretory vesicle
3. The vesicle may travel to the plasma membrane, releasing its contents to the extracellular environment.
Secreted protein
Cell membrane
Extracellular fluid

Golgi Complex (Golgi Apparatus)

- Stacked and flattened membranous sacs (cisternae) with bulbous ends, associated with swarms of tiny vesicles
- There are two "faces" or "sides" of the Golgi: *cis* and *trans*

1. The cis face receives vesicles with membrane and/or lumenal proteins made in RER

   vesicles fuse to the cis face as they come from the ER

2. The trans face buds off vesicles that may move to the plasma membrane to fuse with it, or to become a membranous special endosome or organelle, such as a lysosome or parts of a peroxisome

Golgi Trafficking Models

- Two models are proposed to explain how protein trafficks from cis to trans faces

1. Stationary cisternae
   the cisternae from cis to trans have a special set of enzymes that each perform a different function, and proteins move from cisternae to cisternae via shuttle vesicles
2. Cisternal maturation
   the cisternae move from cis to trans, changing the sets of enzymes within them during the transition to cause changes to the proteins they contain

Golgi Functions

- Membrane proteins in particular get modified extensively in the Golgi
- Oligosaccharides attached to membrane proteins in the ER to become glycoproteins will have those oligosaccharide molecules altered by enzymes in the Golgi
- Some membrane proteins may have mannose molecules phosphorylated, designating these proteins to become part of lysosomes

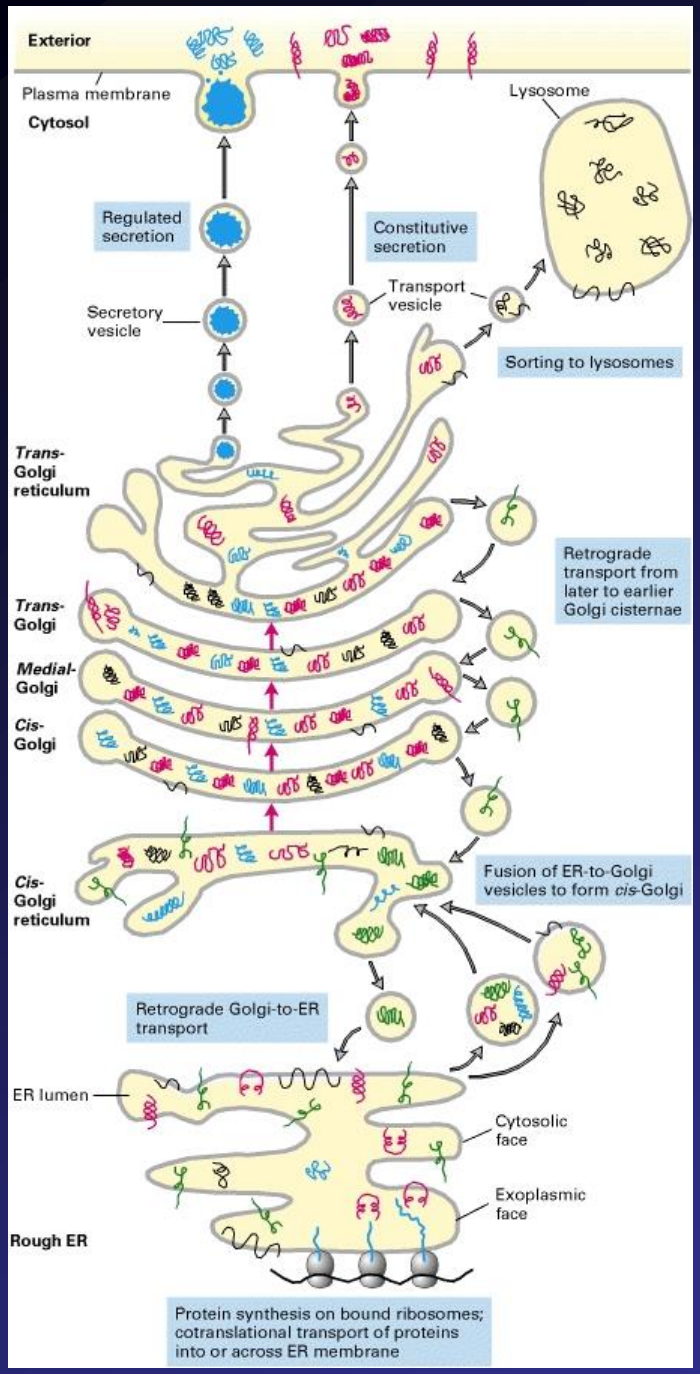
Trafficking Fates 1 of 2

Vesicles budding off the trans Golgi have three fates

1. Constitutive secretion
   - The vesicle goes to the plasma membrane (PM) surface and fuses immediately, providing phospholipids and membrane proteins need on the PM
   - If there are lumenal proteins, they are expelled as needed into the extracellular space to be used
2. Regulated secretion
   - The vesicle often contains in its interior secretory substances that might be proteins and other molecules
   - It moves just inside the PM and waits for a signal to fuse with the PM and expel the contents by exocytosis

Trafficking Fates 2 of 2

3. Lysosomal formation
   The vesicle is to become a lysosome, and has special digestive or degradative proteins contained within its interior that will be activated at the appropriate time it is fused with a vesicle that was created by any of the forms of endocytosis (phagocytosis, pinocytosis, receptor-mediated endocytosis) already discussed
   The lysosome also has H^+-ATPases membrane proteins in particular, which are to pump protons into its interior when activated



This figure nicely shows how vesicles coming off the *trans*-Golgi face can move towards 3 distinctive pathways:

1. constitutive secretion: the vesicles move immediately from the Golgi to the plasma membrane (PM), fuse with it, and allow their membrane to become part of the PM, thus replenishing the PM with phospholipids and membrane proteins it needs; there may also be contents in the vesicle exocytically expelled
2. regulated secretion: vesicles move from Golgi to just next to the PM. Then a signal occurs that usually causes voltage- or ligand-gated Ca^{2+}-channels to open, Ca^{2+} rushes in, and the vesicles fuses and expels its contents exocytically
3. lysosomal formation: these vesicles remain in the cytoplasm and will fuse with phagosomes or other endosomes formed form surface, acidifying the contents and activating digestive enzymes, absorbing the breakdown products

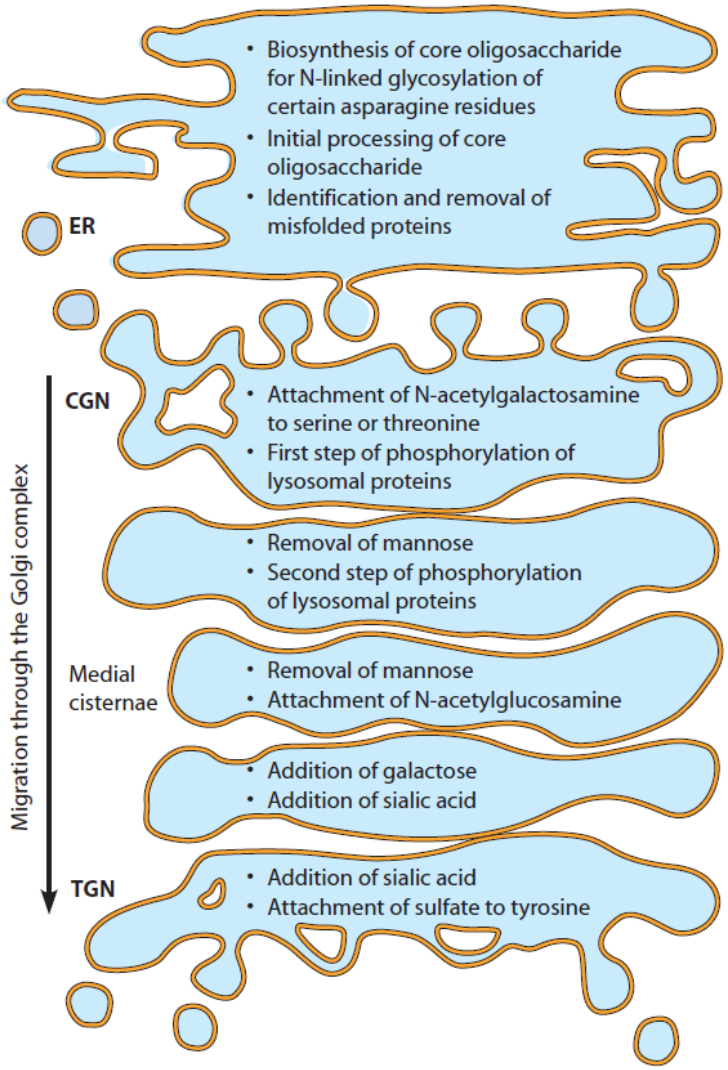


FIGURE 12-6 Compartmentalization of the Steps of Glycosylation and Subsequent Modification of Proteins. Enzymes that catalyze specific steps of glycosylation and further modification of proteins reside in different compartments of the ER and Golgi complex. Processing occurs sequentially as proteins travel from compartment to compartment. The steps listed in the figure are examples of potential modifications and do not necessarily occur with all glycoproteins.

This figure shows details of what biochemical processes occur in the Golgi complex from the cis to the trans face in the maturation of many different types of proteins (particularly glycoproteins) that are fated to be on the plasma membrane or into lysosomes

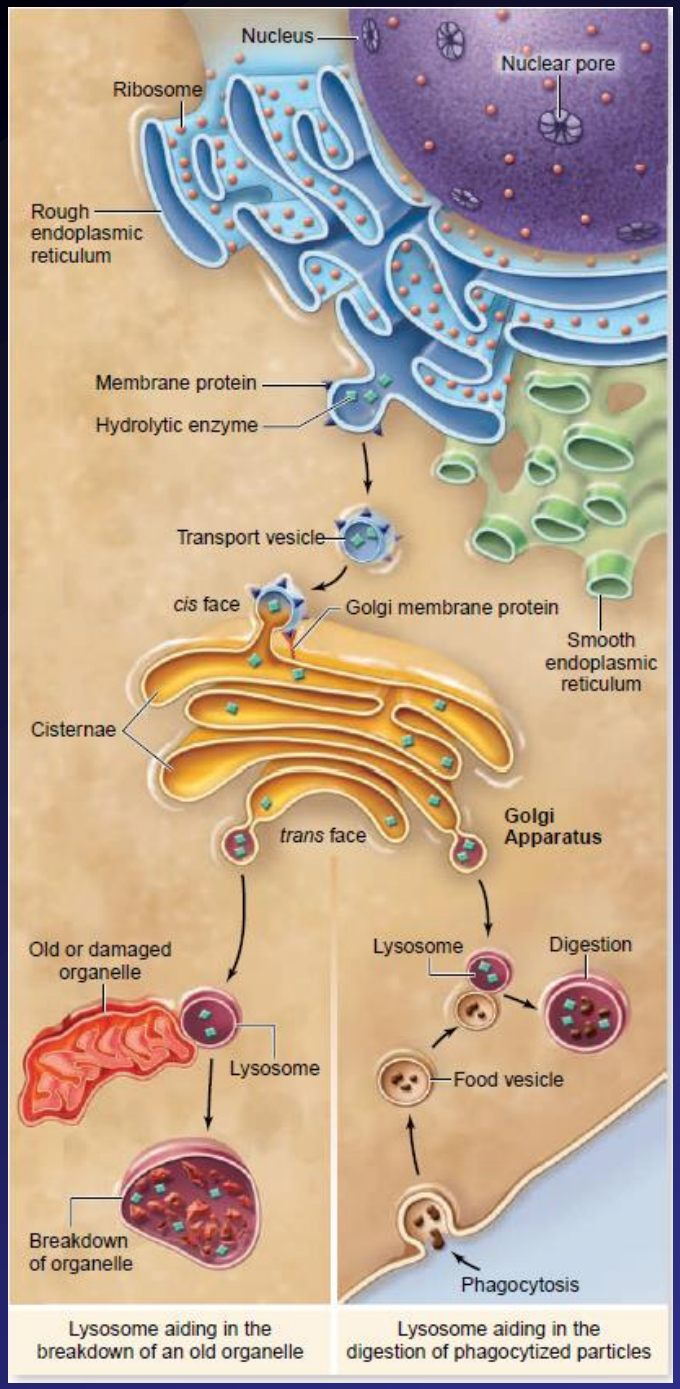
Glycoprotein Formation

- The initial glycosylation of a glycoprotein with its oligosaccharide(s) first occurs within the lumen of the RER
- This initial glycoprotein proceeds to the Golgi: within the Golgi, the oligosaccharide is set upon with enzymes that add more sugar monosaccharides and modify others. These modifications are often to oxidize the terminal monosaccharides of the oligosaccharide that turn it into a negatively charged acidic form, or to put other unusual sugar monosaccharides on the glycoprotein
- As the glycoprotein moves through the Golgi, the oligosaccharide that is modified signals which enzymes are to modify it further to make the glycoprotein have a functional form

Lysosomes

- Born as "endosomes" which contain inactive enzymes, lysosomes ("disintegrator bodies") are spherical membranous organelles containing digestive enzymes
- Lysosomes are large and abundant in phagocytes, immune defense cells that dispose of invading bacteria and cell debris
- Lysosomal enzymes can digest almost all kinds of biological molecules. They work best in acidic conditions, and their digestive enzymes are called acid hydrolases
- The lysosomal membrane is adapted to serve lysosomal functions in two important ways:

1. It contains H^+ (proton) "pumps" membrane proteins, which are ATPases (use energy) that gather hydrogen ions from the surrounding cytosol to maintain the organelle's interior acidic pH
2. The membrane retains the acid hydrolases while permitting the final products of digestion to escape so that they can be used by the cell or excreted. In this way, lysosomes provide sites where digestion can proceed safely within a cell.



- Yet another visual showing the endomembrane system and relationships of the ER, Golgi, plasma membrane, and the vesicles that form and fuse between these cytological structures

Lysosomal Mechanism

- Primary lysosomes emerge from the trans Golgi, and lie dormant until activated
- They are activated by merging with a heterophagic or autophagic vesicles
- This triggers a proton (H^+) pump, which acidifies the lysosomal contents
- Acidification activates proenzymes to change to active enzymes: a pH =5 is about optimal for these enzymes
- Lysosomal enzymes include proteases, amylases, and lipases, which digest the major biomolecular classes: proteins, carbohydrates, lipids
- After digestion, indigestible material may or may not be expelled by exocytosis

Lysosomal Activities

Heterophagy

- Cellular nutrition, metabolic functions, such as glycogen breakdown and release
- Defense: killing bacterial invaders, or removing foreign particles

Autophagy

- Cleaning up and recycling old worn-out organelles
- Autolysis = cellular suicide
- Apoptosis: removal of unwanted cells and tissues during fetal development (such as the webs between the fingers and toes of a developing fetus)
- Extracellular digestion – special lysosome formation releases active enzymes by exocytosis:
  - Acrosome on sperm cells which contains enzymes that break down the outer membrane of the ovum
  - Osteoclasts Breaking down bone to release calcium ions into the blood).

Peroxisomes

- Usually spherical membranous sacs present in all eukaryotic cells but in large number in liver and kidney cells
- Smaller than mitochondria but vary in size
- Not entirely derived from the ER unlike other endomembrane organelles and structures
  - Peroxisomes were believed to increase their numbers by a fission process (bud & separate) not fully understood
  - Recent suggests that most new peroxisomes form by budding off of the endoplasmic reticulum via a special ER machinery that differs from that used for vesicles destined for modification in the Golgi apparatus

Peroxisome Functions 1 of 2

- Peroxisome has its name because it deals with peroxides: such as hydrogen peroxide: H_2O_2

  Hydrogen peroxide can dissociate to form hydroxyl free radicals (•OH), which are extremely chemically reactive with biomolecules like proteins (affecting function) or DNA (potentially causing mutagenesis)
- It has the heme (iron/Fe^{2+})-containing enzyme catalase to detoxify H_2O_2

$$2\ H_2O_2 \rightarrow O_2 + 2\ H_2O$$

- It also has the Zn^{2+}/Cu^{2+}-containing enzyme superoxide dismutase (SOD) to eliminate the very reactive oxygen species, superoxide anion (O_2^-)

$$2\ O_2^- + 2\ H^+ \rightarrow H_2O_2 + O_2$$

Note that SOD produces H_2O_2, which must then be handled by catalase

Peroxisome Functions 2 of 2

- Peroxisomes catabolize (break down) long chain fatty acids (more than 16 carbons) so that mitochondria can catabolize them further, in a process called beta (β)-oxidation
- They also break down unusual amino acids (D-amino acids) and other nitrogen-containing compounds, using oxidative enzymes
- They have oxidative enzymes to detoxify alcohol and aldehydes like formaldehyde & acetaldehyde

Why do peroxisomes generate these reactive oxygen species [ROS] (H_2O_2, superoxide anion)?

- Notice the above processes use words like "oxidation"
- Peroxisomes contain enzymes (oxidoreductases: oxidases, reductases, dehydrogenases) which utilize O_2 to perform catabolic metabolism
- These enzymes often generate ROS purposely or adventitiously as part of the catalysis of trying to move 1 or 2 electrons in oxidation-reduction reactions

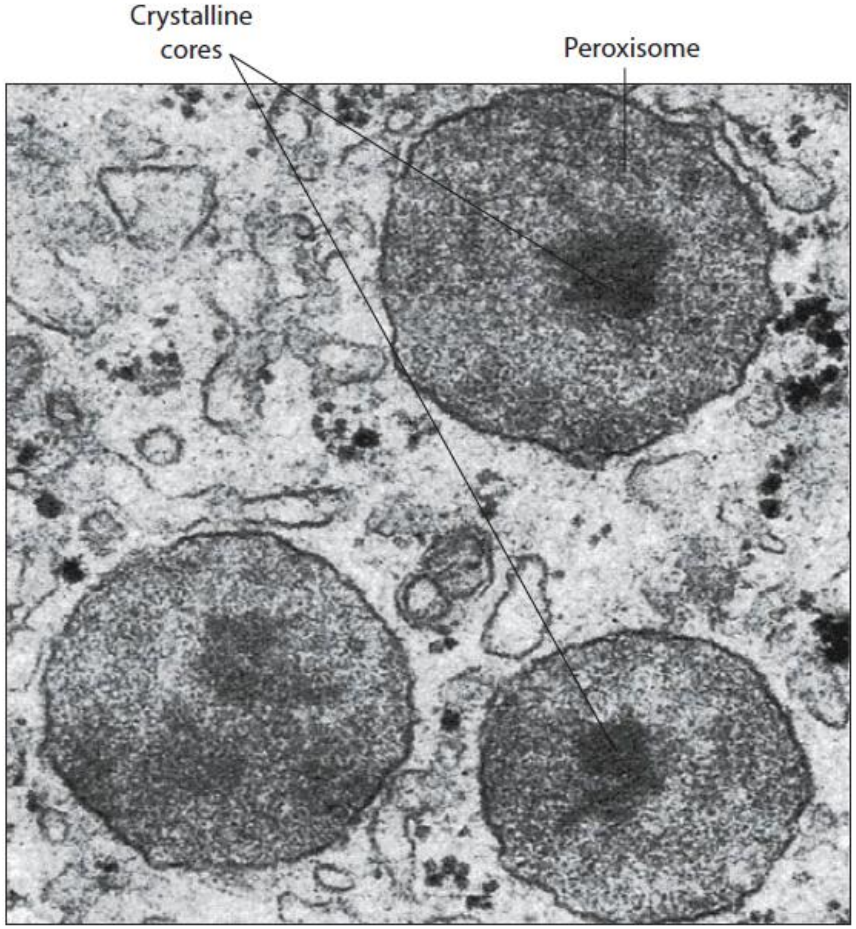


FIGURE 12-23 Peroxisomes in Animal Cells. This electron micrograph shows several peroxisomes (microbodies) in the cytoplasm of a rat liver cell. A crystalline core is readily visible in each microbody. In animal microbodies, the cores are almost always crystalline urate oxidase (TEM).

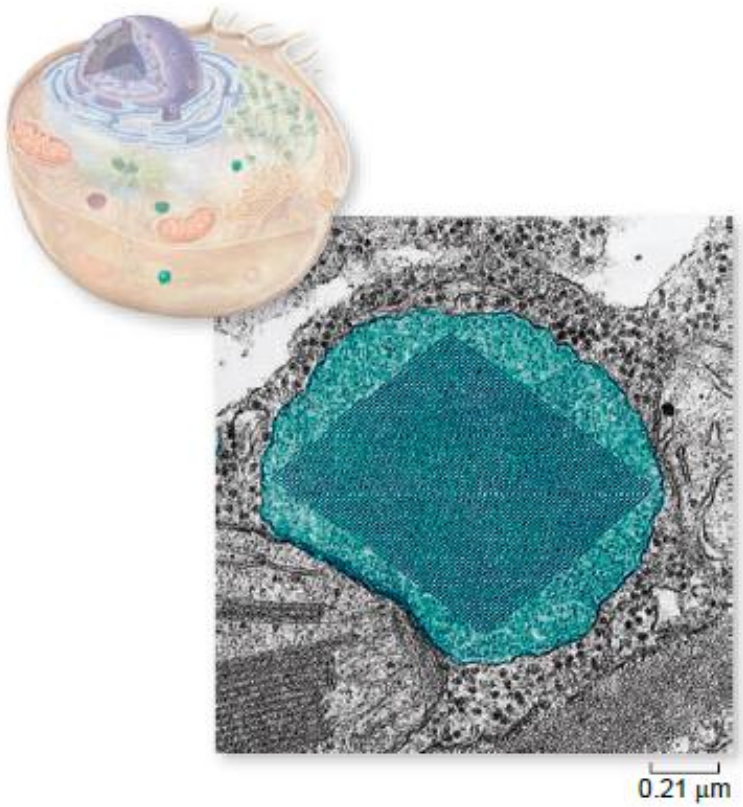


Figure 4.14 A peroxisome. Peroxisomes are spherical organelles that may contain a large crystal structure composed of protein. Peroxisomes contain digestive and detoxifying enzymes that produce hydrogen peroxide as a by-product. A peroxisome has been colored green in the electron micrograph.

Reading (Sources)

- Becker's WotC: pp 324-326, 330-341, 356-358
- Raven: Chap 4.4: pp 69-72
- Marieb: pp 68-71