*Overview of the Endomembrane System*

Parts: ER, golgi complex, endosomes, lysosomes, vacuoles

*Dynamic activities of endomembrane system are highly conserved*
- Materials are shuttled between organelles from the Golgi complex to the plasma membrane in a small, membrane-bound transport vesicles.
  - Transport vesicles:
    o bud from a donor membrane compartment
    o move through the cytoplasm in a directed manner often pulled by major proteins that operate on tracks formed by microtubules and microfilaments of the cytoskeleton
- Distinct pathways that have been studied
- Biosynthetic pathway
  o Proteins are synthesized in the ER, modified during passage through the golgi complex, and transported to various destinations (plasma membrane, lysosome, vacuoles).
- Secretory pathway
  o Many of the proteins synthesized in the ER are destined to be discharged (secreted) from the cell
  o 2 types
    - Constitutive secretion
      • Materials are transported in secretory vesicles from their sites of synthesis and discharged into the extracellular space in a continual manner
      • Engaged by most cells
      • Process that contributes not only to the formation of the extracellular matrix but to the formation of the plasma membrane itself
  o Regulated secretion
    • Materials are stored as membrane-bound packages and discharged only in response to an appropriate stimulus (e.g., endocrine cells that release hormones; pancreatic acinar cells that release digestive enzymes, nerve cells that releases neurotransmitters)
    • Materials to be secreted are stored in large, densely packed, membrane bound secretory granules
- Endocytic pathway
  • Movement of materials from outer surface of cell to compartments such as endosomes and lysosomes located within the cytoplasm.
  • Analogous to movement of trucks carrying different types of cargo along the various highways of the city.
  • Require defined traffic patterns to ensure materials are accurately delivered to the appropriate sites
  • Various types of cargo
    - Secreted proteins
    - Lysosomal enzymes

*Membrane proteins are routed to their appropriate cellular destinations by virtue of specific "addresses" or sorting signals encoded in the amino acid sequence of the proteins or in the attached oligosaccharides.

*Sorting signals are recognized by specific receptors that reside in the membranes or surface coats of budding vesicles (ensures that protein is transported to right destination). Movement of the molecules can be traced through autoradiography or through Green Fluorescent Protein (GFP).*

- **Overview of the Endomembrane System**
  - Pulse: the brief incubation with radioactivity of protein during which labeled amino acids are incorporated into protein
  - Chase: period when tissue is exposed to the unlabeled medium
  - A period during which additional proteins are synthesized using nonradioactive amino acids
  - Longer the chase, farther the radioactive proteins manufactured during the pulse will have traveled from their site of synthesis within the cell
- **Green Fluorescent Protein (GFP)**
  o Utilizes a gene isolated from a jellyfish the amino acid sequence, protein synthesis, specific receptors
  - Pulse: the brief incubation with radioactivity of protein during which labeled amino acids are incorporated into protein
  - Chase: period when tissue is exposed to the unlabeled medium
  - A period during which additional proteins are synthesized using nonradioactive amino acids
  - Longer the chase, farther the radioactive proteins manufactured during the pulse will have traveled from their site of synthesis within the cell
- **Endoplasmic Reticulum**
  - 2 sub compartments: rough ER (RER) and smooth ER (SER)
  - Both have a system of membranes that encloses a space, or lumen, that is separated from the surrounding cytoplasm
  - Both share many of the same proteins and engage in certain common activities (synthesis of certain lipids and cholesterol)
- **RER**
  o Typically composed of a network of flattened sacs (cisternae)
  o Continuous with the outer membrane of the nuclear envelope, which also bears ribosomes on its cytosolic surface
  o Contains proteins involved in the movement of nascent proteins into ER lumen
  o The starting point of biosynthetic pathway
  o RER lumen is packed with molecular chaperones that recognize and bind to unfolded or misfolded proteins and give them the opportunity to attain their correct or native 3D structure
  - Functions
    • Site of protein synthesis, carbohydrate chains and phospholipids that journey through the membrane compartments
    • Include secreted proteins, integral membrane proteins, soluble proteins that reside within compartments of endomembrane system
- **SER**
  o Highly curved and tubular, forming an interconnected system if pipelines traversing the cytoplasm
  o Contains reticulons: membrane-bending proteins which are largely absent form flattened RER sheets; maintains the curvature of SER
  - Functions:
    • Synthesis of steroid hormones in the endocrine cells of the gonad and adrenal cortex
    • Detoxification in the liver (including barbiturates and ethanol, whose chronic use can lead to proliferation of the SER in liver)
    • Sequestration of calcium ions within the cytoplasm of cells

*The regulated release of calcium from SER of skeletal and cardiac muscles cells (sarcoplasmic reticulum; muscle cells) triggers contraction.

Polypeptides are synthesized at two distinct locales within the cell
- One-third of proteins encoded by mammalian genome are synthesized on ribosomes attached to cystolic surface of RER membranes
- Other polypeptides are synthesized on free ribosomes (ribosomes not attached to ER and subsequently released in cytosol
- Includes protein destined to remain in cytosol, peripheral proteins of cystolic surface of membranes, proteins that are transported to the nucleus, peroteins to be incorporated into peroxisomes, chloroplasts, and mitochondria.
Determination of location in a cell where a protein is synthesized (signal hypothesis)
- Site of synthesis of a protein was determined by the sequence of amino acids in the N-terminal portion of the polypeptide (first part to emerge from the ribosome during protein synthesis)
- Secretory proteins contain a signal sequence at the N-terminus that directs the emerging polypeptide and ribosome to the ER membrane
- Polypeptide moves into cisternal space of ER through a protein-lined, aqueous channel in ER membrane

*As the signal sequence emerges from the ribosome, the hydrophobic signal sequence is recognized by a signal recognition particle (SRP)
- SRP: binds signal sequence and ribosome arresting further synthesis of polypeptides
- Bound SRP serves as a tag that enables the entire complex to bind specifically to the cisternal surface of ER membrane

**Cellular membranes are asymmetric:** 2 phospholipid layers if a membrane have different compositions

**Synthesis of Membrane Lipids**
- Most membrane lipids are synthesized entirely within ER
  - Exceptions are: Spingomyelin and glycolipids (synthesis begins in ER and completed in golgi complex; some unique lipids of mitochondrial and chloroplast membranes (synthesized by enzymes that reside in those membranes)

**Golgi Complex**
- Divided into several functionally distinct compartments arranged along an axis from the cis or entry phase closest to ER
- Plays a key role in the assembly of the carbohydrate component of glycoproteins and glycolipids
- Cis-most phase composed of an interconnected network of tubules (CGN)
  - CGN (Cis-Golgi network)
    - Sorting station that distinguishes between proteins to be shipped back to ER
- Medial golgi
  - Intermediary cisternae
- Trans or exit face
  - Found at opposite end of the stack of the golgi apparatus
  - TGN (trans-Golgi network)
    - Sorting station where proteins are segregated into different types of vesicles heading either to the plasma membrane or the various intracellular destination

**Movement of materials through the Golgi complex is facilitated by vesicular transport which is facilitated by “coated vesicles”**

3 best studied coated vesicles
- COPII coated vesicles: move materials from ER forward to the ERGIC
- COP I coated vesicles: move materials in a retrograde direction (from ERGIC and Golgi stack “backward” toward the ER; and from the Golgi cisternae “backward” to the cis-Golgi cisternae
- Clathrin-coated vesicles: move materials from the TGN to endosomes, lysosomes, and plant vacuole; also move materials from plasma membrane to cytoplasmic compartments along the endocytic pathway

Additional terms to remember:
- VSV: vesicular stomatitis virus (used in GFP) *Viruses are useful because they infected cells into factories for the production of viral proteins, which are carried like any other protein cargo through the biosynthetic pathway
- Homogenize: break-up of cells
- Mutant: an organism or cultured cells whose chromosomes contain one or more genes that encode abnormal proteins; in absence of vesicle formation, mutant cells accumulate an expanded ER
- Temperature-sensitive mutants: function normally at a reduced (permissive) temp but not at an elevated (restrictive) temp
- Subcellular fractionation: vesicles derived from different organelles have different properties
- Microsomes: heterogenous collection of similar sized vesicles formed by membranous vesicles derived from endomembrane system primarily ER and golgi complex

**Cell-free systems:** do not contain whole cells; provide a wealth of info about biological processes that were impossible to study within the complex environment of intact cells

**RNA interference (RNAi):** a process in which cells produce small RNAs (siRNAs) that bind to specific mRNAs and inhibit the translation of these mRNAs into proteins
- Translocon: protein-lined channel embedded in ER membrane through which nascent polypeptide is able to move in its passage from the ribosome to ER lumen
- GTP binding proteins (G proteins): play key regulatory roles in many diff cellular processes; like "molecular switches” G protein turns process ON while hydrolysis of G protein turns process OFF
- Signal peptidase: proteolytic enzyme that removes N-terminal portion containing the signal peptide from most nascent polypeptides
- Flippases: lipid molecules that are flipped into opposite leaflet through enzyme actions
- Glycosyltransferases: large family of membrane-bound enzymes that catalyzes addition of sugars to an oligosaccharide chain/ simply catalyze the glycosylation processes in golgi

**THE GOLGI COMPLEX**
- Camillo Golgi
  - Italian biologist
  - Discovered a darkly stained reticular network located near the cell nucleus
    - GOLGI COMPLEX
- Golgi Complex
  - Has a characteristic morphology consisting of flattened, disk-like, membranous cisternae with dilated rims and associated vesicles and tubules
  - Cisternae
    - Diameter typically 0.5µm to 1.0µm
    - Arranged in orderly stack and are curved
  - Golgi stack
    - Typically contains fewer than 8 cisternae
    - An individual cell may contain several distinct stacks, depending on the cell type
    - Mammalian cells
      - Golgi stacks are interconnected to form a single, large ribbon-like complex situated adjacent to the cell’s nucleus
  - Divided into several functionality distinct compartments arranged along an axis from cis (entry face closest to ER) to the trans (exit face at the opposite end of the stack)
    - cis-most face of the organelle
      - Composed of interconnected network of tubules (cis Golgi network - CGN)
        - CGN → sorting station that distinguishes between proteins to be shipped back to ER and those allowed to proceed to the next Golgi station
      - Composed of a series of large flattened cisternae
        - cis cisternae
        - medial cisternae
        - trans cisternae
    - trans-most face of the organelle
      - Contains a distinct network of tubules and vesicles (trans Golgi network - TGN)
        - TGN → sorting station where proteins are segregated into different types of vesicles heading either to the plasma membrane or to various intracellular destination
  - The membranous elements of the Golgi complex are thought to be supported mechanically by a peripheral membrane skeleton or scaffold composed of a variety of proteins, including members of the spectrin, ankyrin, and actin families
  - The Golgi scaffold may be physically linked with motor proteins that direct the movement of vesicles and tubules entering and exiting the Golgi complex.
  - A separate group of fibrous proteins are thought to form a Golgi “matrix,” that plays a key role in the disassembly and reassembly of the Golgi complex during mitosis.
Golgi complex plays a key role in the assembly of the carbohydrate component of glycoproteins and glycolipids. Most of the mannose residues are also removed from the core oligosaccharides and other sugars are added sequentially by various glycosyltransferases. The sequence in which sugars are incorporated into oligosaccharides is determined by the spatial arrangement of the specific glycosyltransferases that come into contact with the newly synthesized protein as it moves through the Golgi stack (Golgi Complex – RER).

- Sialytransferases (enzyme)
  - Places sialic acid at the terminal position of the chain in animal cells
  - Localized in the trans face of the Golgi stack
- Glycosylation in the ER
  - Assembles a single core oligosaccharide
  - Produce carbohydrate domains remarkable sequence diversity
- The Golgi complex is also the site of synthesis of most of a cell’s complex polysaccharides
  - Glycosaminoglycan chains of the proteoglycan
  - Pectins and hemicellulose found in the cell walls of plants

Movement of Material through the Golgi Complex

- Up until mid-1980s
  - Golgi cisternae were transient structures
  - Golgi cisternae formed at the cis face of the stack
  - Fusion of the membranous carriers from the ER and ERGIC
  - Each cistern physically moved from the cis to the trans end of the stack
  - Cisternal Maturation Model
    - Each cistern “matures” into the next cisterna along the stack
- Mid-1980s to mid-1990s
  - The cisternae of a Golgi stack remain in place as stable compartments
  - Vesicular Transport Model Cargo
    - Cargo is shuttled through the Golgi stack, from the CGN to the TGN, in vesicles that bud from one membrane compartment and fuse with a neighboring compartment farther along the stack
    - Each of the various Golgi cisternae of a stack has a distinct population of resident enzymes
    - Large numbers of vesicles can be seen in electron micrographs to bud from the rims of Golgi cisternae
- Consensus shifted back to the cisternal maturation model
  - The cisternal maturation model envisions a highly dynamic Golgi complex in which the major elements of the organelle, the cisternae, are continually being formed at the cis face and dispersed at the trans face
  - Certain materials that are produced in the endoplasmic reticulum and travel through the Golgi complex can be shown to remain within the Golgi cisternae and never appear within Golgi-associated transport vesicles

Types of Vesicle Transport and Their Functions

- The biosynthetic pathway of a eukaryotic cell consists of a series of distinct membrane-bound organelles that function in the synthesis, modification, and delivery of soluble and membrane proteins to their appropriate destination in the cell.
- The dark-staining layer consists of a protein coat formed from soluble proteins that assemble on the cytosolic surface of the donor membrane at sites where budding takes place
  - Each coated bud pinches off to form a coated vesicle
- Functions of protein coats
  - Act as a mechanical device that causes the membrane to curve and form a budding vesicle
  - Provide a mechanism for selecting the components to be carried by the vesicle
    - Cargo consisting of secretory, lysosomal, and membrane proteins to be transported
    - Machinery required to target and dock the vesicle to the correct acceptor membrane
- Vesicle coat is composed of two distinct protein layers:
  - An outer cage or scaffolding that forms the framework for the coat
  - An inner layer of adaptors that binds both to the outer surface of the lipid bilayer and the membrane’s cargo
- Three best studied coat vesicle
  - COPI-coated vesicles
    - Move materials from the ER “forward” to the ERGIC and Golgi complex
  - COPI-coated vesicles
    - Move materials in a retrograde direction
      - From the ERGIC and Golgi stack “backward” toward the ER and
      - From trans Golgi cisternae “backward” to cis Golgi cisternae
  - Clathrin-coated vesicles
    - Move materials from the TGN to endosomes, lysosomes, and plant vacuoles
    - Move materials from the plasma membrane to cytoplasmic compartments along the endocytic pathway
    - Trafficking from endosomes and lysosomes.

COPII-Coated Vesicles: Transport Cargo from the ER to the Golgi Complex

- COPII-coated vesicles mediate the first leg of the journey through the biosynthetic pathway—from the ER to the ERGIC and Golgi complex
- COPII coats select and concentrate certain components for transport in vesicles
- Certain integral membrane proteins of the ER are selectively captured because they contain "ER export" signals as part of their cytosolic tail.
  - Signals interact specifically with COPII proteins of the vesicle coat.
- Proteins selected by COPII-coated vesicles include:
  - Enzymes that act at later stages in the biosynthetic pathway, such as the glycosyltransferases of the Golgi complex.
  - Membrane proteins involved in the docking and fusion of the vesicle with the target compartment.
- Membrane proteins that are able to bind soluble cargo - Cells lacking a specific cargo receptor typically fail to transport a specific subset of proteins from the ER to the Golgi complex.

- Sar1
  - Small G protein
  - Recruited specifically to the ER membrane
  - Regulatory role
    - Initiating vesicle formation
    - Regulating the assembly of vesicle coat

COPI - Coated Vesicles: Transporting Escaped Proteins Back to the ER
- COPI-coated vesicles accumulate in the presence of a nonhydrolyzable GTP analogue because, similar to their COPII counterparts, the coat contains a small membrane-bending GTP-binding protein, called Arf1, whose bound GTP must be hydrolyzed before the coat can disassemble.
- COPI-coated vesicles have been most clearly implicated in the retrograde transport of proteins, including the movement of:
  - Golgi resident enzymes in a trans-to-cis direction
  - ER resident enzymes from the ERGIC and the Golgi complex back to the ER

Retaining and Retrieving Resident ER Proteins
- Studies suggest that proteins are maintained in an organelle by a combination of two mechanisms:
  - Retention of resident molecules that are excluded from transport vesicles.
    - Retention may be based primarily on the physical properties of the protein.
  - Retrieval of "escaped" molecules back to the compartment in which they normally reside.

- Proteins that normally reside in the ER contain short amino acid sequences at their C-terminus that serve as retrieval signals, ensuring their return to the ER if they should be accidentally carried forward to the ERGIC or Golgi complex.
  - The retrieval of "escaped" ER proteins from these compartments is accomplished by specific receptors that capture the molecules and return them to the ER in COPI-coated vesicles.

Beyond the Golgi Complex: Sorting Proteins at the TGN
- The trans Golgi network (TGN)
  - Last stop in the Golgi complex
  - Functions as a major sorting station, directing proteins to various destinations
- The best understood of the post-Golgi pathways is one that carries lysosomal enzymes

Sorting and Transport of Lysosomal Enzymes
- Lysosomal proteins are synthesized on membrane-bound ribosomes of the ER and carried to the Golgi complex along with other types of proteins.
  - Once in the Golgi cisternae, soluble lysosomal enzymes are specifically recognized by enzymes that catalyze the two-step addition of a phosphate group to certain mannose sugars of the N-linked carbohydrate chains.
Lysosomal enzymes possess phosphorylated mannose residues, which act as sorting signals. Lysosomal enzymes carrying a mannose 6-phosphate signal are recognized and captured by mannose 6-phosphate receptors (MPRs), which are integral membrane proteins that span the TGN membranes. Lysosomal enzymes are transported from the TGN in clathrin-coated vesicles. Coats of the vesicles contain:
- An outer honeycomblike lattice composed of the protein clathrin, which forms a structural scaffold,
- An inner shell composed of protein adaptors, which covers the surface of the vesicle membrane that faces the cytosol.
Lysosomal enzymes are escorted from the TGN by a family of adaptor proteins called GGAs.

Clathrin-coated vesicles that bud from the TGN contain GGA, an adaptor protein consisting of several distinct domains. One of the GGA domains binds to the cytosolic domains of membrane proteins, including those that will ultimately reside in the boundary membrane of the lysosome and also the MPR that transports lysosomal enzymes. Other GGA domains bind to Arf1 and to the surrounding cytosolic network of clathrin molecules.

**Sorting and Transport of Nonlysosomal Proteins**
- Vesicle fusion requires specific interactions between different membranes
  - Vesicles from the ER
    - Fuse with the ERGIC or cis Golgi network and not with a trans cistern
- Selective fusion is one of the factors that ensures a directed flow through the membranous compartments of the cell.
- It is thought that a vesicle contains specific proteins associated with its membrane that govern the movements and fusion potential of that vesicle.
- Steps that occur between the stages of vesicle budding and vesicle fusion
  - Movement of the vesicle toward the specific target compartment.

Membranous vesicles must move considerable distances through the cytoplasm before reaching their eventual target. These types of movement are mediated largely by microtubules, which act like railroad tracks carrying cargo containers along a defined pathway to a predetermined destination.

**Tethering vesicles to the target compartment.**
- The initial contacts between a transport vesicle and its target membrane, such as a Golgi cisterna, are thought to be mediated by a diverse collection of “tethering” proteins.
- Two groups of tethering proteins have been described:
  - Rod-shaped, fibrous proteins
    - Capable of forming a molecular bridge between the two membranes
  - Large multiprotein complexes
    - Appear to hold the two membranes in closer proximity
- Different tethering proteins initiate fusion between different types of membranes.
- Golgins
  - One class of fibrous tethering proteins
  - Act in and around the Golgi complex
  - May serve as “tentacles” to reach out and capture transport vesicles carrying Golgi-bound cargo.
- Rabs
  - Small G proteins
  - Confer the specificity between vesicle and target membrane
  - Cycle between an active GTP-bound state and an inactive GDP-bound state. GTP-bound
  - Associate with membranes by a lipid anchor
  - Constitute the most diverse group of proteins involved in membrane trafficking.
  - Associated with different membrane compartments.

**Docking vesicles to the target compartment.**
- The membranes of the vesicle and target compartment come into close contact with one another.
- The key proteins that engage in these interactions are called SNAREs.
- All contain a segment in their cytosolic domain called a SNARE motif that consists of 60–70 amino acids capable of forming a complex with another SNARE motif.
- SNAREs can be divided functionally into two categories
  - v-SNAREs which become incorporated into the membranes of transport vesicles during budding
  - t-SNAREs which are located in the membranes of target compartments.

**Fusion between vesicle and target membranes**
- When artificial lipid vesicles (liposomes) containing purified t-SNAREs are mixed with liposomes containing purified v-SNAREs, the two...
Lysosomes

- An animal cell's digestive organelles
- A typical lysosome contains at least 50 different hydrolytic enzymes produced in the rough ER and targeted to these organelles
- Lysosomal enzymes can hydrolyze virtually every type of biological macromolecule
- All have their optimal activity at an acid pH and thus are called acid hydrolases.
  - The pH optimum of these enzymes is suited to the low pH of the lysosomal compartment, which is approximately 4.6
- Lysosomal membranes also contain a variety of highly glycosylated integral proteins
- Carbohydrate chains are thought to form a protective lining that shields the membrane from attack by the enclosed enzymes.
- The appearance of lysosome in electron micrographs is neither distinct nor uniform
- The best studied role of lysosomes is the breakdown of materials brought into the cell from the extracellular environment

Autophagy

- Lysosomes also play a key role in organelle turnover
  - The regulated destruction of the cell's own organelles and their replacement
- Autophagy
  - Process
  - An organelle is surrounded by a double membrane structure to produce a double-membrane-sequestering vesicle called an autophagosome
  - The outer membrane of the autophagosome fuses with a lysosome, generating a structure called an autolysosome, in which both the inner membrane of the autophagosome and the enclosed contents are degraded.
  - The products of these degradative reactions are made available to the cell.
  - It is estimated that 1 to 1.5 percent of the proteins within a healthy liver cell are degraded via autophagy per hour as part of a normal process of cellular renewal.
  - Autophagy probably evolved in early eukaryotic organisms as a response to nutrient deprivation.
  - If a population of cells is placed under starvation conditions, a marked increase in autophagy is observed.
  - Under these conditions, cells acquire energy to maintain their life by cannibalizing their own organelles.
  - Deletion of certain of these autophagy genes can have serious consequences for embryonic development or adult physiology of model organisms
  - Autophagy helps protect an organism against intracellular threats ranging from abnormal protein aggregates to invading bacteria and parasites.
  - Autophagy has also been implicated in the prevention of neurodegeneration.
  - Autophagy may even play a role in the prevention of certain types of cancers and slowing the body's aging process.
  - Once the digestive process in the autolysosome has been completed, the organelle is termed a residual body.
    - Depending on the type of cell, the contents of the residual body may be eliminated from the cell by exocytosis, or they may be retained within the cytoplasm indefinitely as a lipofuscin granule.

THE HUMAN PERSPECTIVE: DISORDERS RESULTING FROM DEFECTS IN LYSOSOMAL FUNCTIONS

- Mannose 6-phosphate residues in lysosomal enzymes act as an "address" for delivery of these proteins to lysosomes.
- The discovery of the lysosome address was made in studies of patients with a rare and fatal inherited condition known as Gaucher disease.
  - Many cells in these patients contain lysosomes that are bloated with undegraded materials.
  - The secreted enzymes lacked the mannose phosphate residues that are present on the lysosomal enzymes of cells from normal individuals.
  - The 1-cell defect was soon traced to the deficiency of an enzyme (N-acetylgalactosamine phosphate transferase) required for mannose phosphorylation in the Golgi complex.
- In 1965, H. G. Hers of the University of Louvain in Belgium offered an explanation as to how the absence of α-glucosidase could lead to the development of a fatal inherited condition known as Pompe disease.
  - In the absence of α-glucosidase, undigested glycogen accumulated in lysosomes
    - Causing swelling of the organelles and irreversible damage to the cells and tissues.
- Lysosomal storage disorders
  - Characterized by the deficiency of a single lysosomal enzyme and the corresponding accumulation of undegraded substrate.
The symptoms of lysosomal storage diseases can range from very severe to barely detectable, depending primarily on the degree of enzyme dysfunction.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Enzyme deficiency</th>
<th>Glycosidase Storage Diseases</th>
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<tbody>
<tr>
<td>Gaucher’s disease</td>
<td>beta-galactosidase</td>
<td>Gaucher’s disease</td>
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<tr>
<td>Tay-Sachs disease</td>
<td>beta-hexosaminidaseA</td>
<td>Hexosaminidase A</td>
</tr>
<tr>
<td>Fabry’s disease</td>
<td>alpha-galactosidase</td>
<td>Fabry’s disease</td>
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<tr>
<td>Niemann-Pick disease</td>
<td>alpha-glucosidase</td>
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- **Tay-Sachs disease**
  - Results from a deficiency of the enzyme \( \beta-N-hexosaminidase A \), an enzyme that degrades the ganglioside GM2
  - GM2 is a major component of the membranes of brain cells, and in the absence of the hydrolytic enzyme, the ganglioside accumulates in the bloated lysosomes of brain cells, causing dysfunction.
  - In its severe form, the disease is characterized by progressive mental and motor retardation, as well as skeletal, cardiac, and respiratory abnormalities.
  - 1 in 3600 newborns among Jews of eastern European ancestry.

- **Gaucher’s disease**
  - A deficiency of the lysosomal enzyme glucocerebrosidase, which can be alleviated by enzyme replacement therapy.
  - Infants with Gaucher’s disease accumulate large quantities of glucocerebrosides in the lysosomes of their macrophages, causing spleen enlargement and anemia.

- **Substrate reduction therapy**
  - Small molecular weight drugs are administered to inhibit the synthesis of the substances that accumulate in the disease.

12.7 Plant Cell Vacuoles
- Single membrane-bound, fluid-filled central *Vacuole*
- Storing solutes, macromolecules (ions, sugars, amino acids, proteins and polysaccharides) & toxic compounds for injury caused by herbivore (cyanide containing glycosides and glucosinolates).
- Isolate products of *metabolic reactions*.
- Tonoplast – membrane that surrounds the vacuole, containing number of active transport systems that pump ions into vacuolar compartment to a concentration much higher than that in the cytoplasm or extracellular fluid.
- High ion concentration – H2O enters vacuole (osmosis).
- Hydrostatic turgor pressure by vacuole – provides mechanical support, stretches cell wall during cell growth.
- Sites of intracellular digestion, plant vacuoles have *acid hydrolases*.
- pH of vacuole is maintained at low value by V-type *H+ - ATPase*.
- Acid hydrolases.

**Endocytic Pathway: Moving Membrane and Materials into the Cell Interior**
- **Endocytosis** – cell internalizes cell-surface receptors and bound extracellular ligands.
- **Phagocytosis** – describes the uptake of particulate matter.
- **Endocytosis**
  - Bulk-phase (pinocytosis) – non-specific uptake of extracellular fluids, large or small.
  - Receptor-mediated uptake of specific extracellular macromolecules (ligands) following their binding to receptors on the external surface of the plasma membrane.
  - Receptor-Mediated Endocytosis and the Role of Coated Pits (PM – Plasma Membrane)
    - Means for selective and efficient uptake of macromolecules.

- Have receptors for diff. types of ligands (hormones, growth factors, enzymes and blood borne proteins carrying nutrients).
- Substances that enter a cell by means of clathrin-mediated RME become bound to receptors that collect in specialized domains of the PM, known as *coated pits*.
  - Coated pits – sites where surface is indented and PM is covered on its cytoplasmic face by clathrin.
  - Invaginate into cytoplasm and pinch free of the PM to form coated vesicles.
  - Coated pit – derived from structure of clathrin building blocks.
- Each clathrin coat mol. consists of three heavy chains and three light chains, joined together at the center to form a three-legged assembly called *triskellion*.
- Coated vesicles that form during endocytosissent contain layer of adaptors between clathrin lattice and surface of the vesicle facing the cytosol.
- AP2 – best studied adaptor, incorporated into the vesicles that bud from the PM contain multiple subunits having different functions.
- The subunit of AP2 adaptors engages the cytoplasmic tails of PM receptors leading to the concentration of these selected receptors and their bound cargo molecules into emerging coated vesicle
- In contrast, B-adaptin subunit of AP2 adaptors binds and recruits the clathrin molecules of the overlying lattice.
- COP I and clathrin coated vesicles have outer geometric scaffold and inner layer of adaptor proteins.
- Outer geometric scaffolds – subunits of clathrin lattice overlap, COP II lattice do not overlap.
- Vertex of COP I coat formed by four edges rather than 3 in clathrin.
- Dynamin – large GTP-binding protein required for release of clathrin-coated vesicle from membrane on w/c it forms.
- Self-assemblies into helical collar around neck of invaginated coated pit.
- Hydrolysis of the bound GTP by polymerized dynamin mol. induces twisting motion in the dynamin helix that severs the coated vesicle from the plasma membrane.
- Acts as an enzyme capable of utilizing the chemical energy of GTP to generate mechanical forces.
- **ATPase Hsc70** – helps in dissoc. of clathrin coat from vesicle recycled by clathrin coat by a cofactor, auxilin.

The Role of Phosphoinositides in the Formation of Coated Vesicles
- Phosphate groups can be added to diff. positions of the sugar ring of the Phosphatidylinositol (PI), converting them into phosphoinositols (Pi).
- Phosphorylated rings of these phosphoinositides reside at the surface of the membrane to be recognized and bound by particular proteins.
- Diff. phosphoinositides concentrated in diff. membrane compartments = unique “surface identity”.
- PI(P,4,5)P2 has dynamic regulatory role bec. can be rapidly formed and destroyed enzymes that are localized at particular places & times within the cell.

**Endocytic Pathway**
- Route of molecules taken into a cell by endocytosis.
- Receptors subjected to endocytosis:
  - "Housekeeping receptors" – resp. for uptake of materials used by the cell
    - Ex. Transferrin, Low density lipoprotein – mediate delivery to cells of iron and cholesterol respectively
  - "Signaling receptors" – binding extracellular ligands that carry messages that change the activities of the cell.
- Ligands include insulin, growth factors (EGF) that bind to surface receptor and signal a physiologic response inside the cell.
- Endocytosis of 1st group of receptors leads to delivery of the bound materials (iron and cholesterol), to the cell and return of the receptor to the cell surface for uptake.
- Endocytosis of 2nd group of receptors often leads to the destruction of the receptor, a process called **receptor down-regulation**, which has the effect of reducing the sensitivity of the cell to further stimulation by the hormone or growth factor.
- Receptor down-regulation is a mechanism by w/c cells regulate their ability to respond to extracellular messengers.
- Signaling receptors – endocytosis and destruction by covalent attachment of a tag to cytoplasmic tail of the receptor while residing at cell surface.
- Tag – small protein called ubiquitin.
- Following internalization, vesicle-bound materials transported to endosomes w/c represent distribution centers.
- Fluid in the lumen is acidified by H+ - ATPase in the boundary membrane.
- Endosomes divided into two classes:
  o Early – near the peripheral of cell
  o Late – closer to nucleus “multivesicular bodies (MVB)”
- Receptors taken up by endocytosis transported in vesicles to an early endosome – serves as sorting station that directs diff. types of receptors and ligands along diff. pathways.
- “Housekeeping receptors” dissoc. from bound ligands as result of high H+ concentration of early endosomes.
- Receptors concentrated into specialized tubular compartments of early endosome, represent recycling centers. Vesicles that bud from these tubules carry receptors back to the PM for addtl rounds of endocytosis.
- In contrast, released ligands become concentrated into a sorting compartment before being dispatched to a late endosome and before dispatched to a late endosome and to a lysosome where final processing occurs.
- “Signaling receptors” w/ ubiquitin tags do not recycle back to membrane.
- Ubiquitinylated receptors are sequestered into pop. of small, spherical vesicles that crowd the interior of late endosome.
- Vesiculation process (4 diff. ESCRT complexes act in seq.
  1. sort ubiquitinylated receptors into a cluster w/in late endosomal membrane
  2. cause patch of membrane to invaginate as bud into lumen of late endosome
  3. sever neck of the invagination to release newly formed intraluminal vesicle

**LDLs and Cholesterol Metabolism**
- Provides animal cells w/ exogenous cholesterol (part of PM, precursor of steroid).
- Cholesterol – hydrophobic mol. transported in blood as part of hugh lipoprotein complexes “low-density lipoprotein”
- Each LDL contains central core of 1500 cholesterol mol esterified to long-chain fatty acids.
- Core surrounded by single layer of phospholipids w/ 1 copy of large protein called apolipoprotein B-100 (binds to LDL)
- LDL transported to PM, concentrated in coated pits, even absence of LDL ligand.
- Receptors ready to take up blood-borne lipoproteins
- Once LDL particles bound to coated pit, pit invaginates to form coated vesicle, clathrin coat disassembled and LDL receptors pass through early endosomes and back to PM.
- Meanwhile, LDL particles are delivered to late endosomes and lysosomes, where protein component is degraded and cholesterol is deesterified and used by cell in membrane assembly or other metabolic processes.
- Niemann-Pick type C disease – lack one proteins req. to transfer to cholesterol out of lysosomes.
- Resulting accumulation of cholesterol lead to nerve degeneration and death during early childhood.
- LDL level in blood rel. to atherosclerosis – formation of plaques in inner lining of arteries that reduce flow of blood through the vessel and act as sites for formation of blood clots.
- Blood clots that block coronary arteries lead to myocardial infarction (heart attack).
- Lowering LDL levels through statins the block HMG CoA reductase (synthesis of cholesterol) Lower blood cholesterol – risk of heart attack is reduced
- HDLs (high-density lipoproteins) contain diff. protein (apolipoprotein A-I) play diff. physiologic role in body
- Carries cholesterol in opposite direction
- Excess cholesterol transporte dout of PM of body cells circulating HDL particles, w/c carry cholesterol to liver for extraction
- LDL serves to carry cholesterol mol from liver, synthesized packaged through the blood to the body’s cells.
- High levels of HDL assoc. with decreased risk (good cholesterol)
- Small molecular weight CETP inhibitors increase HDL levels
- PCSK9 – proteolytic enzyme that destroys LDL receptors in liver.
- Reduced LDL cholesterol levels decreased heart disease

**Phagocytosis**
- Uptake of large particles from environment
- Single cell protists (amoebas and ciliates)
- Trap food particles and smaller organisms, enclosing in folds in PM
- Fuse to produce vacuole (phagosome) pinchess inwardly from PM
- Phagosome fuses w/ lysosome, digested w/in resulting phagolysosome
- In animals, protective mechanism “professional” phagocytes
  - Macrophages and neutrophils
  - Wander through blood and tissues phagocytizing invading pathogens, damaged and dead cells and debris
- Recognized and bound by receptor on surface of phagocyte prior to uptake
- Once inside phagocyte, microorganisms killed by lysosomal enzymes or oxygen free radicals generated w/in lumen of phagosome.
- Driven by contractile activities of actin-containing microfilaments that underlie the plasma membrane
- Not all bacteria ingested by phagocytic cells are destroyed.
- Some hijack phagocytic machinery to survive
- Mycobacterium tuberculosis, (tuberculosis) taken into cytoplasm of macrophase by phagocytosis, bacterium is able to inhibit fusion of phagosome w/ lysosome.
- Phagosome becomes highly acidic, bacterium appears able to maintain pH despite lowering pH of surrounding medium.
- Cosiella burnetii enclosed in phagosome fuse w/ lysosome but acidic environment nor lysosomal enzymes cannot destroy it.
- Listeria monocytogenes, causes meningitis, produces proteins that destroy integrity of the lysosomal membrane, allowing bacterium to escape into cell’s cytosol.

**POSTTRANSLATIONAL UPTAKE OF PROTEINS BY PEROXISOMES, MITOCHONDRIA AND CHLOROPLAST**
- The division of the contents of a cell into large numbers of compartments presents many organizational challenges to the cell’s protein-trafficking machinery.
- Protein trafficking within a eukaryotic cell
  o Sorting signals, such as the signal peptide of secreted proteins or mannose-phosphate groups of lysosomal enzymes.
  o Receptors that recognize these signals and deliver proteins containing them to the proper compartment.
- Four of the cell’s major organelles—nucleus, mitochondria, chloroplasts, and peroxisomes—import proteins through one or more outer boundary membranes.

**Uptake of Proteins into Peroxisomes**
- Peroxisomes are very simple organelles having only two subcompartments
  o The boundary membrane
  o The internal matrix
- Proteins destined for a peroxisome possess a peroxisomal targeting signal, either a PTS for a peroxisomal matrix protein or an mPTS for a peroxisomal membrane protein.
- Peroxisomes are somehow able to import peroxisomal matrix proteins in their native, folded conformation, even those that consist of several subunits.

**Uptake of Proteins into Mitochondria**
- Mitochondria have four subcompartments into which proteins can be delivered:
  o An outer mitochondrial membrane (OMM)
  o Inner mitochondrial membrane (IMM)
  o Intermembrane space
  o Matrix
- Roughly 99 percent of the organelle’s proteins are encoded by the nuclear genome, synthesized in the cytosol, and imported posttranslationally.
- Mitochondrial proteins contain signal sequences that target them to their home base.
- The polypeptide is targeted to a mitochondrion by a targeting sequence, which is located at the N-terminus in the matrix protein and is located internally in most inner membrane proteins. Cytosolic Hsp70 molecules unfold the...
polypeptides prior to their entry into the mitochondrion. The proteins are recognized by membrane receptors (red transmembrane proteins) and translocated through the OMM by way of pores in the TOM complex of the OMM. Most integral proteins of the IMM are directed to the TIM22 complex of the IMM, which steers them into the lipid bilayer of the IMM. Mitochondrial matrix proteins are translocated through the TIM23 complex of the IMM. Once the protein enters the matrix, it is bound by a mitochondrial chaperone, which may either pull the polypeptide into the matrix or act like a Brownian ratchet to ensure that it diffuses into the matrix. Once in the matrix, the unfolded protein assumes its native conformation with the help of Hsp60 chaperone. The presequence is removed enzymatically.

**Uptake of Proteins into Chloroplast**

- Chloroplasts have six subcompartments into which proteins can be delivered:
  - Inner envelope membrane
  - Outer envelope membrane
  - Intervening intermembrane space
  - Stroma
  - Thylakoid membrane
  - Thylakoid lumen

- Chloroplast and mitochondrial import mechanisms exhibit many similarities, although their translocation machineries have evolved independently
  - The vast majority of chloroplast proteins (approximately 3000 in higher plants) are imported from the cytosol,
  - The outer and inner envelope membranes contain distinct translocation complexes (Toc and Tic complexes, respectively) that work together during import
  - Chaperones aid in the unfolding of the polypeptides in the cytosol and folding of the proteins in the chloroplast
  - Most proteins destined for the chloroplast are synthesized with a removable N-terminal sequence (termed the *transit peptide*) that is highly variable in length and sequence.

- Proteins encoded by nuclear genes are synthesized in the cytosol and imported through protein-lined pores in both membranes of the outer chloroplast envelope. Proteins destined for the stroma contain a stroma targeting domain at their N-terminus, whereas proteins destined for the thylakoid contain both a stroma-targeting domain and a thylakoid-transfer domain at their N-terminus. Stromal proteins remain in the stroma following translocation through the outer envelope and removal of their single targeting sequence. The presence of the thylakoid transfer domain causes thylakoid proteins to be translocated either into or completely through the thylakoid membrane. A number of the proteins of the thylakoid membrane are encoded by chloroplast genes and synthesized by chloroplast ribosomes that are bound to the outer surface of the thylakoid membrane.