01.30.09
Lecture 7 - Intracellular compartments and transport II
SNAREs are proteins that target vesicles to specific compartments

v-SNAREs are on vesicles

t-SNARES are on target compartments
SNARE proteins are important for membrane fusion

- v-SNAREs and t-SNAREs bind tightly
- Complexes bring the two membranes together to promote fusion
Review of protein translocation

- Proteins delivered directly from cytosol:
  - Gated transport
  - Transmembrane transport

- Proteins delivered in vesicles:
  - Vesicular transport
Exocytosis & endocytosis

1. The secretory pathway: how newly made lipids, proteins, and carbohydrates are delivered to the cell surface via exocytosis

2. Endocytic pathways: how cells take up fluids and particles (large and small) from the extracellular environment
Most (or all) proteins are covalently modified in the ER

1. Formation of disulfide bonds between cysteine residues of the same polypeptide (intra-molecular) or different polypeptides (inter-molecular)

2. Addition of short oligosaccharide side chains (glycosylation)
Formation of disulfide bonds occurs within the ER

- Oxidation of pairs of cysteine side chains
- Reaction can’t occur in the cytoplasm
Example: antibodies
Many proteins are glycosylated in the ER

1. Polypeptide is translocated into ER lumen
2. Pre-formed, branched oligosaccharide (14 sugars) transferred en bloc from dolichol (a specialized lipid) to specific sequence
3. Sequence contains asparagine - N-linked
4. Reaction catalyzed by oligosaccharide protein transferase (active site oriented towards lumen)
Many proteins are glycosylated in the ER
Some proteins are retained in the ER

- Proteins containing **ER retention signal** are held in the ER lumen (i.e. for disulfide bond formation, oligosaccharide transferases)

- Proteins that are misfolded or fail to oligomerize are held for quality control by **chaperonins**
The Golgi apparatus is made of stacked, flattened membranes.
Proteins are further modified in the Golgi apparatus

• Proteins enter the Golgi via vesicle fusion with the cis face and pass through the Golgi between successive stacks by transport vesicles

• Oligosaccharide chains added in the ER are modified by enzymes in the Golgi - addition and removal of sugars to make complex oligosaccharides

• Reactions are processive - early-acting enzymes in the cis compartments, late acting enzymes in the trans compartments
2 pathways for exocytosis: regulated and constitutive
Exocytosis occurs via 2 distinct pathways: regulated and constitutive

- Constitutive exocytosis: A steady stream of delivery occurring in all cells. Plasma membrane components to replace endocytosed material and for membrane growth.

- Regulated exocytosis: Operates only in cells specialized for secretion (i.e. secretory cells in the gut and glands). **Secretory vesicles** are docked at the plasma membrane until the cells receive an outside signal.
Secretory vesicles package concentrated protein aggregates

- Proteins in this pathway have special surface properties
- The lumen of the TGN is acidic and has high calcium \( \Rightarrow \) aggregation
The endocytic pathway:

• Two main mechanisms of endocytosis:

• Phagocytosis: “cellular eating”; The ingestion of large particles (i.e. microorganisms, cellular debris) via large vesicles called phagosomes. Only occurs in specialized cells

• Pinocytosis: “cellular drinking”; The ingestion of fluid and small molecules via small (<150 nm diameter) vesicles. Occurs in all cells.
Phagocytic cells ingest large particles
Phagocytic cells ingest large particles
Receptor-mediated endocytosis is a specialized form of pinocytosis

- Pinocytosis traps molecules in the extracellular fluid randomly

- Receptor-mediated endocytosis traps specific molecules, concentrates them in vesicles

- Both processes use clathrin-mediated vesicle formation
Endosomes: “sorting stations” for membrane trafficking

- Early endosomes (beneath the membrane) vs. late endosomes (in the center of the cell)

- Lumen is kept acidic by ATP-driven proton pump - many (not all) ligands dissociate from their receptor

- Receptors may be recycled, degraded, or undergo transcytosis
Example: receptor-mediated endocytosis of LDL
Different receptors have different fates

- Some receptors are recycled to the same plasma membrane domain
- Some are degraded in lysosomes
- Some are recycled to a different domain (transcytosis)
- Ligands that stay bound in endosome will follow their receptor
Intracellular digestion occurs in lysosomes

- Very acidic (to protect the cell)
- ~40 types of hydrolytic enzymes (acid hydrolases)
- Resident proteins are protected by high levels of glycosylation
Autophagy: a degradation pathway to dispose of obsolete parts of the cell
There are multiple pathways to the lysosome