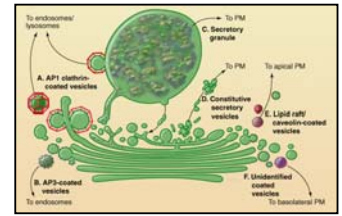


Vesicular traffic Endocytosis

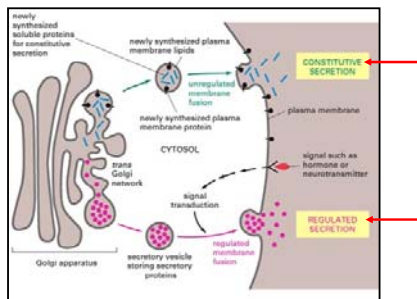


Divergence of cargo at the trans-Golgi network

- Now from Golgi there are many choices...
- Sorting based on
 - Protein motifs
 - Physical properties such as aggregation
 - Geometric consideration



Transport to plasma membrane - exocytosis

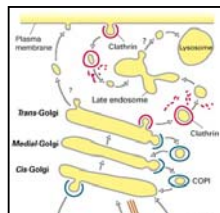


Constitutive secretion

- “Unregulated”, constant
- Whatever was not retained goes to the surface
- No signal necessary
- No known coat proteins have been identified
- Membrane tubules rather than vesicles carry cargo to the surface

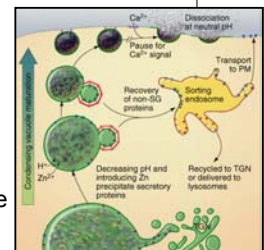
Life after Golgi

- Some proteins emerging from trans Golgi need additional sorting in endosomes - lysosomes before going to the surface
- For proteins that are not needed all the time
 - Hormones
 - Transmitters
 - Cytokines



Regulated secretion

- Sorting controlled by selective protein aggregation with selectogranins in trans-Golgi
- In sorting pathway they can be readdressed to the membrane or destroyed if no longer needed



Endocytosis

- Process of internalization of extracellular molecules
 - Nutrients
 - Termination of signaling
 - Destruction of invaders
- Some toxins, bacteria, viruses and protozoa use it to enter a cell
 - Opportunistic endocytic ligands



Endocytosis

- Internalized molecules enter in membrane vesicles
- Most molecules enter through **receptor mediated endocytosis**
 - Bind to high affinity receptors on the surface (specific, however opportunistic ligands can fool them)
 - Receptor-ligand complexes are internalized



Receptor-mediated endocytosis in clathrin coated vesicles

- Most common type of receptor-mediated endocytosis
- Based on the selective binding between protein to be internalized and receptor on the cell surface
- The way to sort internalized molecules



Receptor-mediated endocytosis in clathrin coated vesicles

- Used to
 - Obtain nutrients (cholesterol, iron)
 - Terminate signaling by removal of signaling molecule (hormones, growth factors)
 - Also recycle synaptic vesicles at presynaptic endings



Ligands that enter by receptor mediated endocytosis

- Nutrients
 - Cholesterol through LDL receptor
 - Iron with carrier protein transferrin



Termination of signaling by receptor mediated endocytosis

- Hormones and growth factors to terminate signaling
 - Insulin
 - Catecholamines
 - Prolactin
 - Growth hormone
 - Epidermal growth factor
 - Nerve growth factor



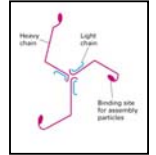
Opportunistic ligands that enter by receptor mediated endocytosis

- Toxins
 - Diphtheria toxin
 - Pseudomonas toxin
 - Cholera toxin
- Viruses
 - Rous sarcoma virus
 - Vesicular stomatitis virus
 - Adenovirus



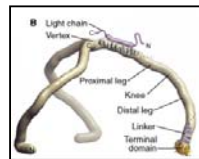
Clathrin

- A coat protein
- Forms special shape called triskelion
 - 3 heavy chains
 - 1 or 2 light chains associated with each heavy chain



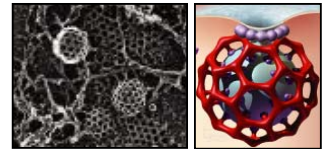
Clathrin

- Heavy chains are rigid and form a cage type structure
 - Distant side contains the globular domain for binding assembly particles
- Light chains are attached near the center
 - Stabilize the structure



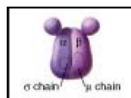
Clathrin assembly

- Triskelions assemble into a cage like structure
- Fibrous network of pentagons and hexagons
- Can self-assemble and form empty cages

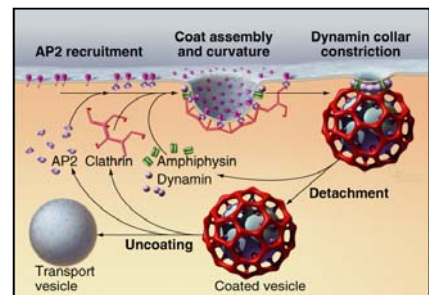


Adapter and assembly proteins

- Assembly is directed by adapter and assembly proteins
 - Adaptins
- Responsible for specificity of trafficking



Formation of endocytic vesicles



Formation of endocytic vesicles

- Binding of the extracellular molecule (ligand) to specific receptor on the cell surface
- Receptor clustering into coated pits
 - Recruitment of adapter proteins
 - Coat formation
- Vesicle budding and pinching
- “Uncoating”



Formation of endocytic vesicles

- Delivery into endosomes (targeting and fusion)
- The receptor - ligand complex gets dissociated or enzymatically degraded and proteins broken down and utilized by the cell



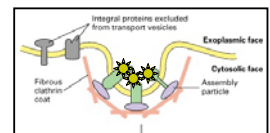
Receptor clustering

- All endocytosed receptors have internalization motifs in the cytoplasmic domains
- These motifs are recognized by adapter proteins that in turn recruit coats
 - Adapter proteins are regulated by GTPases



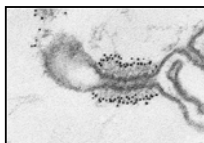
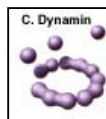
Formation of a clathrin-coated vesicle

- Cytosolic domains of activated receptors bind to adapter proteins
- Adapter proteins bind to clathrin as it polymerizes spontaneously over the region of membrane



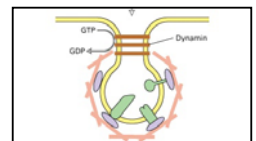
Dynamin and vesicle budding

- Dynamin
 - Globular cytosolic protein
 - GTPase - binds and hydrolyzes GTP
 - Self assembles into a collar



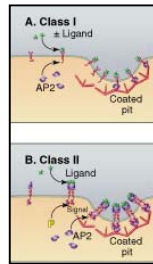
Vesicle budding

- Dynamin polymerizes over the neck of the vesicle and pinches the neck off
 - Energy is coming from the hydrolysis of GTP
- Once the vesicle has formed, the clathrin coat is lost
 - The uncoating uses energy
- Depolymerized clathrin is recycled



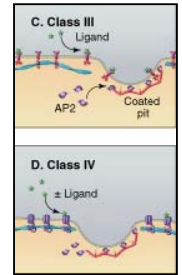
Rates of clathrin mediated endocytosis

- Class I
 - LDL and transferrin
 - Constitutive (no ligand necessary)
- Class II
 - Insulin and EGF
 - Active upon ligand binding



Rates of clathrin mediated endocytosis

- Class III
 - CD4
 - Tethered to cytoskeleton
 - Ligand binding triggers release and endocytosis
- Class IV
 - Resident plasma membrane pumps
 - Tethered to cytoskeleton
 - Internalize slowly if at all



Afterlife of the vesicle

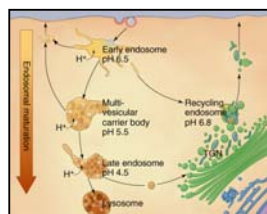
- After the coat is lost, the vesicles join with other vesicles to form an **endosome**

Endosomes

- Major sorting organelles
- 4 classes based on biochemical markers
 - Early or sorting
 - Recycling
 - Multivesicular
 - Late

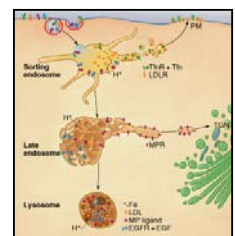
Protein sorting in endosomal pathway

- Endosomes and lysosomes are acidic vesicles
 - Vacuolar V type H^+ pump
- Sorting is pH dependent
- pH gradient through endosomal pathway



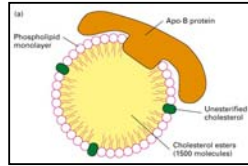
Protein sorting in endosomal pathway

- Ligand is released from the receptor-ligand complex in specific pH
- Ligand stays in the endosome
- Receptor stays in the membrane and is recycled to plasma membrane



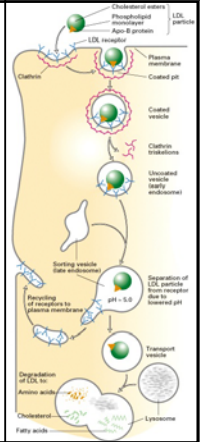
Receptor mediated endocytosis of cholesterol

- LDL particle (bad cholesterol) contains thousands of cholesterol molecules in a phospholipid vesicle bound to Apo-B lipoprotein



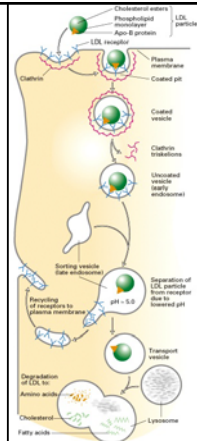
Receptor mediated endocytosis of LDL

- LDL particle binds to the receptor
- Receptor clustering and clathrin assembly
- Vesicle budding
- Clathrin disassembly
- Early endosome fuses with the sorting vesicle



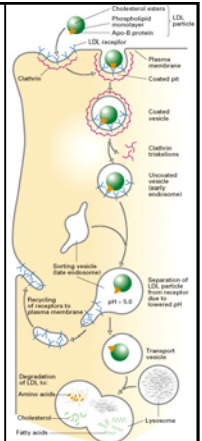
Receptor mediated endocytosis of LDL

- pH drops to about 5.0 - the structure is called a late endosome
- Acidic pH causes the breakdown of receptor-ligand complex
 - Receptors stay in the membrane, ligand is released



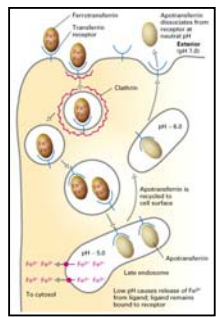
Receptor mediated endocytosis of LDL

- The receptor rich region buds off and recycles to the membrane
- The rest merges with acidic lysosome where the degradation proceeds



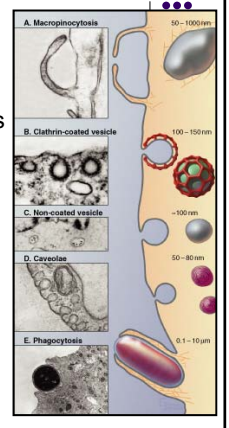
Endocytosis of transferrin-bound iron

- Only iron dissociates from the receptor
- Carrier protein (ligand for the receptor) remains bound to receptor and recycled back to the membrane



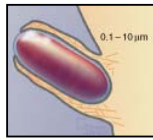
Types of endocytotic vesicles

- Use different coats or no coats
- Have different sizes
- Have different mechanisms of forming



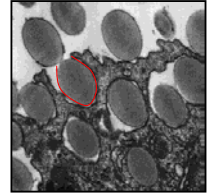
Phagocytosis

- Used mostly in defense mechanisms
- Landmark of mobile cells
 - Macrophages and neutrophils
- Receptor mediated
- Receptor-ligand complex actively triggers particle ingestion



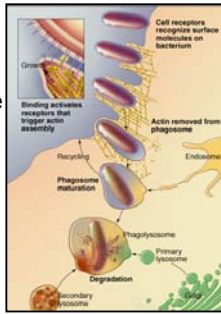
Phagocytosis

- Sometimes ligands have to be delivered to the offending particle
 - In host defence response opsonins (antibodies, complement) bind to foreign particle and mark it for uptake
 - “Zipper” effect between membrane and opsonins



Mechanism of phagocytosis

- Ligand binding activates actin polymerization and amoeba like extensions of the membrane around the particle
- Formation of phagosome
- Phagosome maturation
- Degradation



Types of endocytotic vesicles

- Remember there are other types of vesicles!!!

