

Chapter 2: Exocytosis and endocytosis

Exocytosis and endocytosis

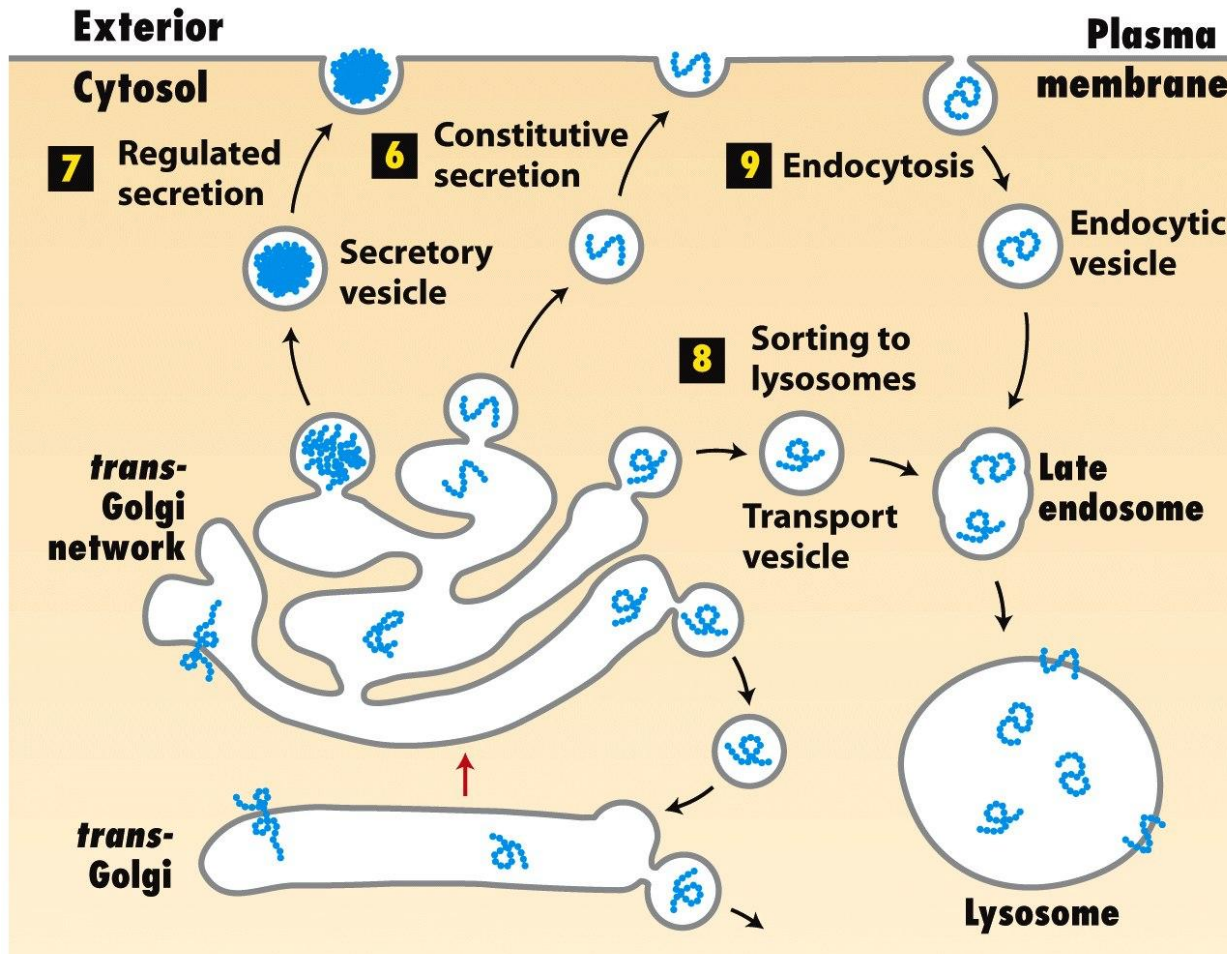
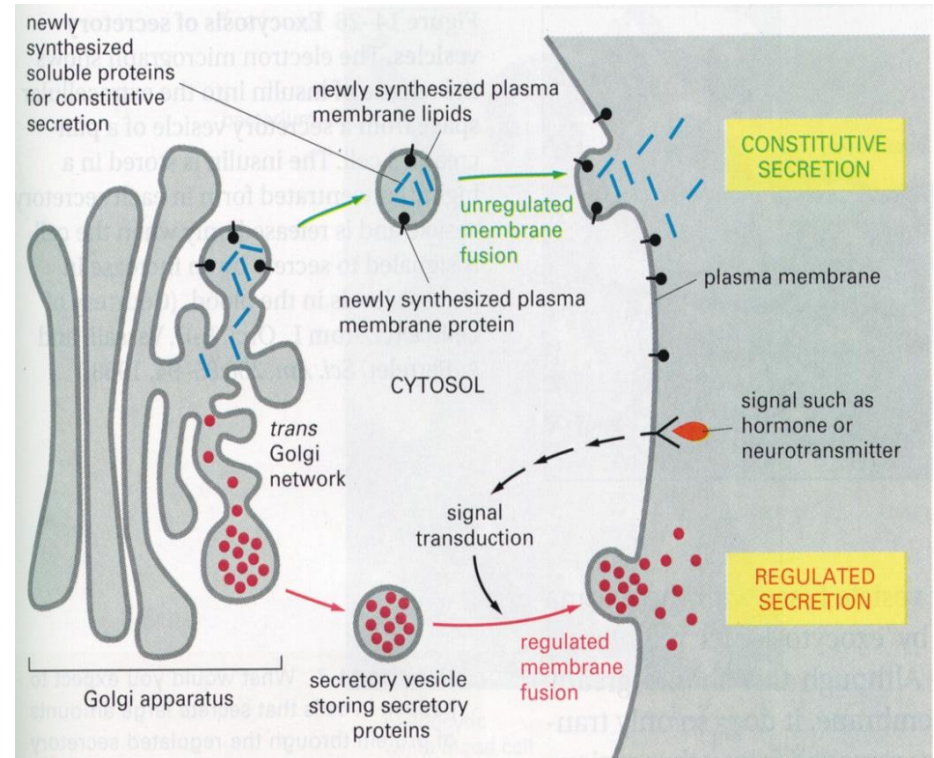


Figure 14-1 part 3
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Transport from the trans-golgi network to the cell exterior: exocytosis.

Two different types of vesicles move proteins from the *trans*-Golgi network to the cell surface: **regulated transport vesicles**, (secretory vesicles), and unregulated transport vesicles (constitutive secretory vesicles). All eukaryotic cells continuously secrete certain proteins, a process called **constitutive secretion**.

Specialized secretory cells also store other proteins in vesicles and secrete them only when triggered by a specific stimulus (**regulated secretion**) such as hormone or neurotransmitter.



Common mechanisms appears to sort regulated proteins into regulated secretory vesicles (recombinant insulin segregate with ACTH in pituitary tumor cells) despite proteins share no identical amino acid sequences that might serve as a sorting sequence

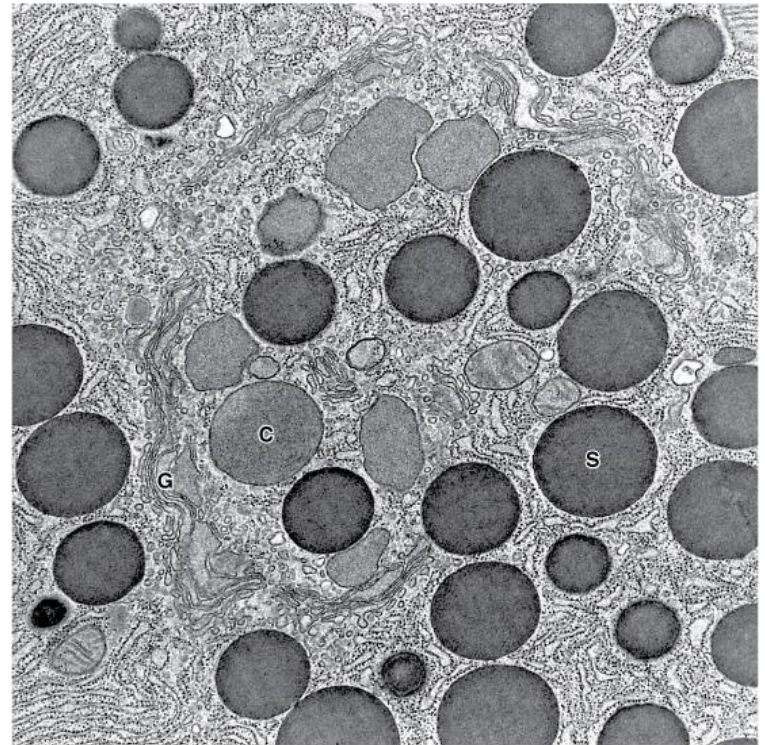
Protein Aggregation in the *Trans-Golgi* May Function in Sorting Proteins into regulated pathway

Sorting into the regulated pathway is controlled by **selective protein aggregation**.

Immature vesicles just budded from the *trans-Golgi network* contain diffuse aggregates of secreted proteins (see figure)

Aggregates have been also found in budding vesicles (condensing vacuoles) indicating that proteins destined for regulated secretory vesicles selectively aggregate together before their incorporation into the vesicles.

Regulated secreted proteins in mammalian cells always contains 3 proteins: **chromogranin A**, **chromogranin B**, and **secretogranin II** that together form aggregates at pH ≈ 6.5 and 1 mM Ca^{2+} (conditions that occur in the TGN) and have a role for sorting of these proteins into regulated secretory vesicles.

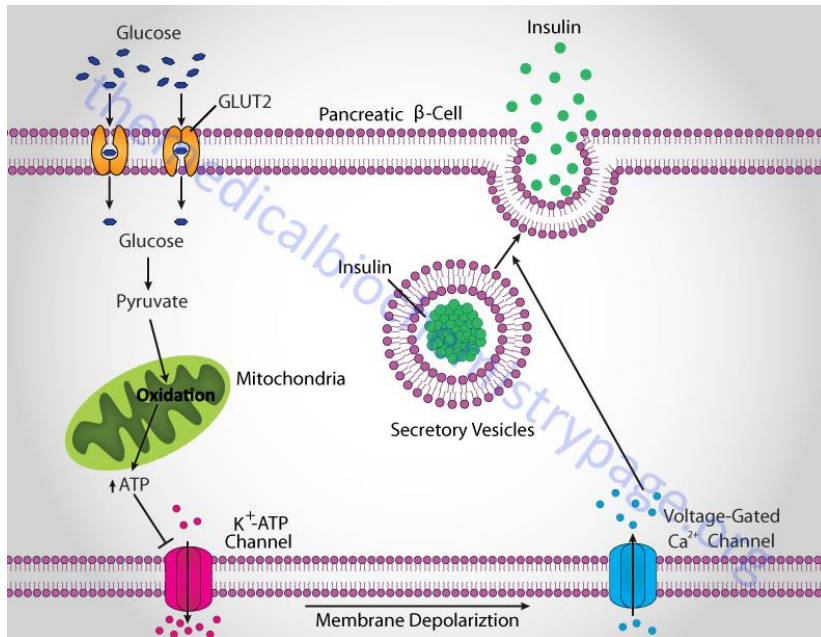


Source: Mescher AL: *Junqueira's Basic Histology: Text and Atlas, 12th Edition*: <http://www.accessmedicine.com>
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TEM of one area of a pancreatic acinar cell shows numerous mature, electron-dense **secretory granules** (S) in association with condensing vacuoles (C) of the Golgi apparatus (G).

Mechanism of glucose-stimulated insulin secretion

Example of regulated secretion: pancreatic cells store newly made insulin in special secretory vesicles and secrete insulin in response to an elevation in blood glucose



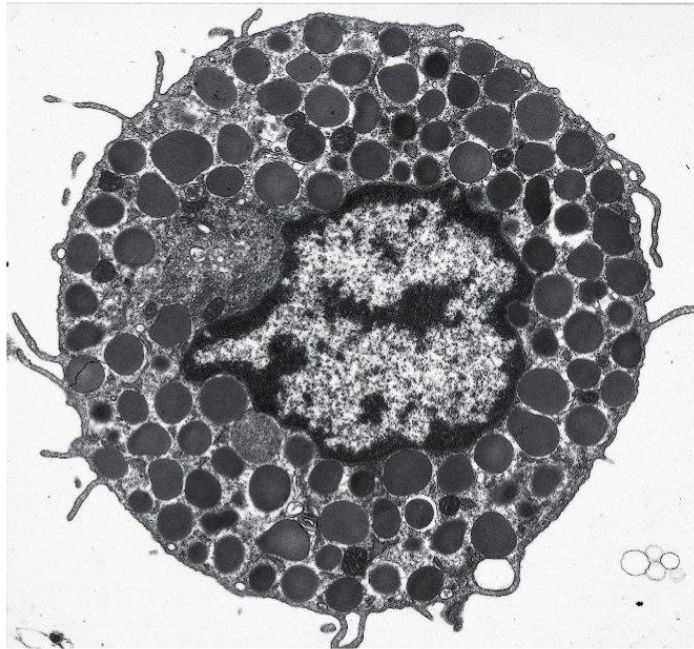
<https://themedicalbiochemistry.org/insulin.php>

A glucose "sensor" mechanism, a metabolic coupling to potassium channels to control plasma membrane potential and a voltage dependent Ca^{2+} channel are required to link blood glucose levels to insulin secretion.

Insulin containing granules are found in a reserve pool and a "readily released" pool.

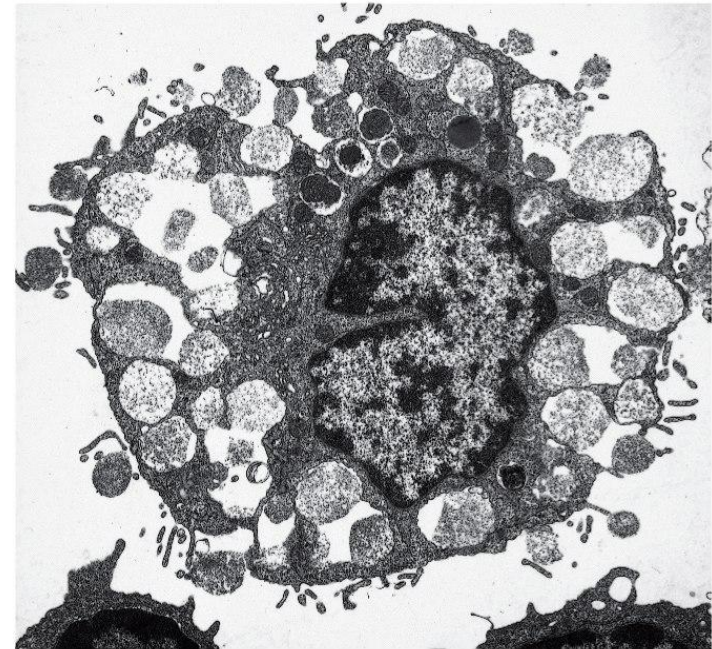
In nerve terminations, the initial signal exocytosis is also an electric excitation (an action potential) that open voltage dependent Ca^{2+} channel which in turn activate the **synaptotagmin** , a SNARE-regulative protein

Electron micrographs of exocytosis in rat mast cells



(A)

5 μ m



(B)

Figure 13-68 Molecular Biology of the Cell 5/e (© Garland Science 2008)

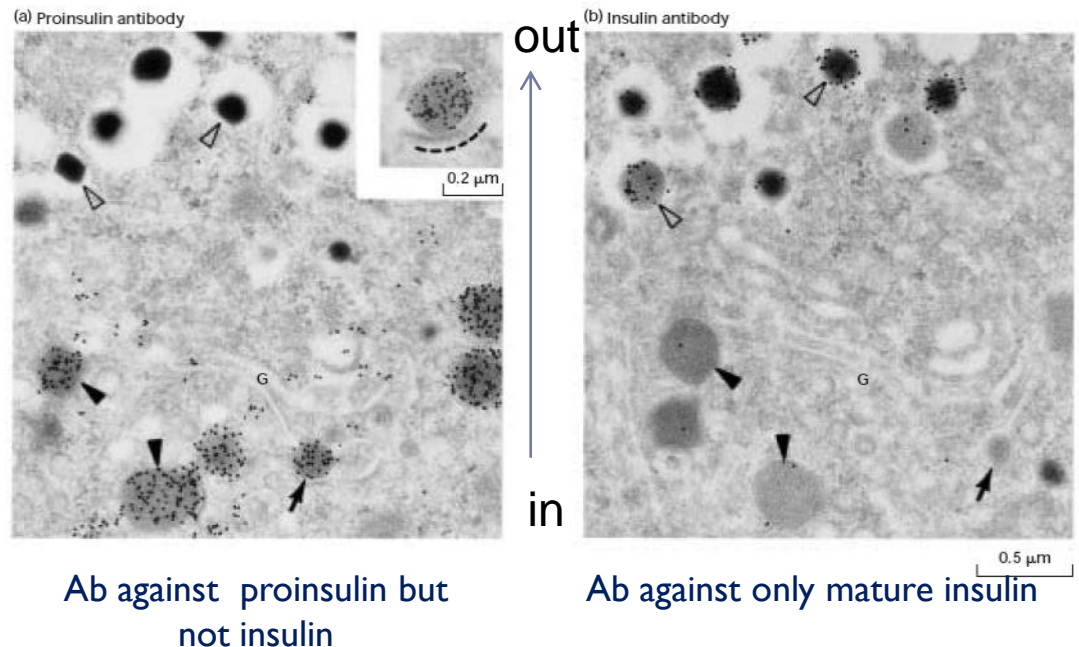
Massive exocytosis (degranulation) of histamine from secretory granules in mast cells.



Proteolytic cleavage of some proproteins, occurs in vesicles after leaving the trans-Golgi network

Some membrane and many soluble secretory proteins are synthesized as relatively long-lived, **inactive precursors**, termed **proproteins (proenzymes)**, that require further proteolytic processing to generate the mature, active proteins. Examples: insulin, glucagon, serum albumin, lysosomal enzymes

Delaying the activation of lysosomal proenzymes until they reach the lysosome prevents them from digesting macromolecules in earlier compartments of the secretory pathway.



The presence of **mature insulin** is visible in secretory vesicles (granules) but not in TGN

Multiple cleavages of the single polypeptide chain yields the chains of mature insulin

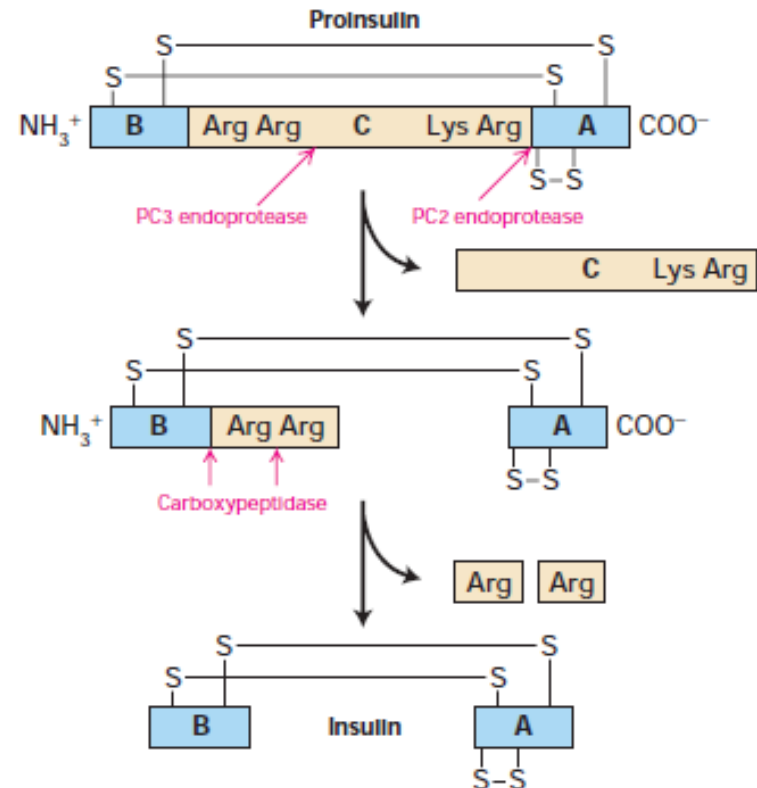
Proinsulin: multiple cleavages of the single polypeptide chain yields the N-terminal **B chain** and the C-terminal **A chain** of mature insulin, which are linked by disulfide bonds.

Responsible proteases: a family of mammalian endoproteases all of which cleave a protein chain on the C-terminal side of an **Arg-Arg** or **Lys-Arg** sequence.

Two endoproteases: PC2 and PC3, are present only in the regulated secretory vesicles, act on precursors of several proteins.

The final processing of many such proteins is catalyzed by a carboxypeptidase

(b) Regulated secreted proteins



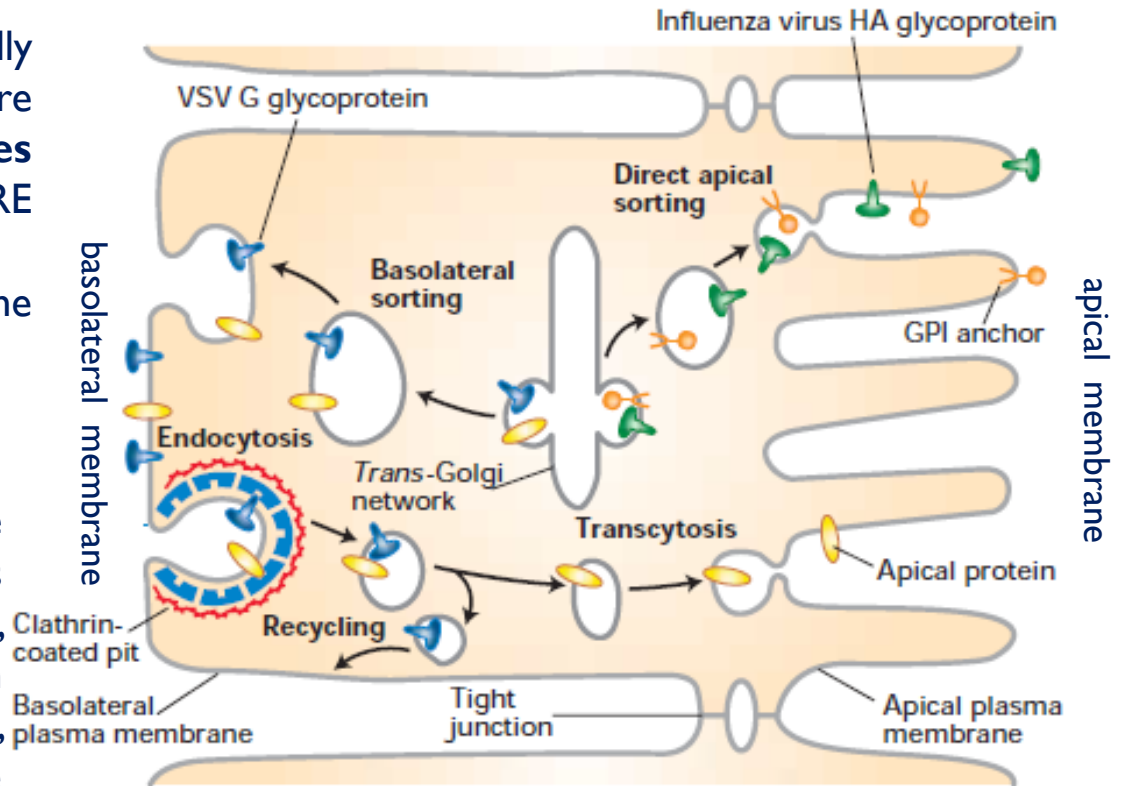
Several Pathways Sort Membrane Proteins to the Apical or Basolateral Region of Polarized Cells

The plasma membrane of **polarized epithelial cells** is divided into **apical** and **basolateral** membranes with distinct sets of proteins. Secreted proteins have to be sorted differentially.

Both sets of proteins are initially located within TGN and are transported **by different vesicles** including distinct Rab and v-SNARE proteins.

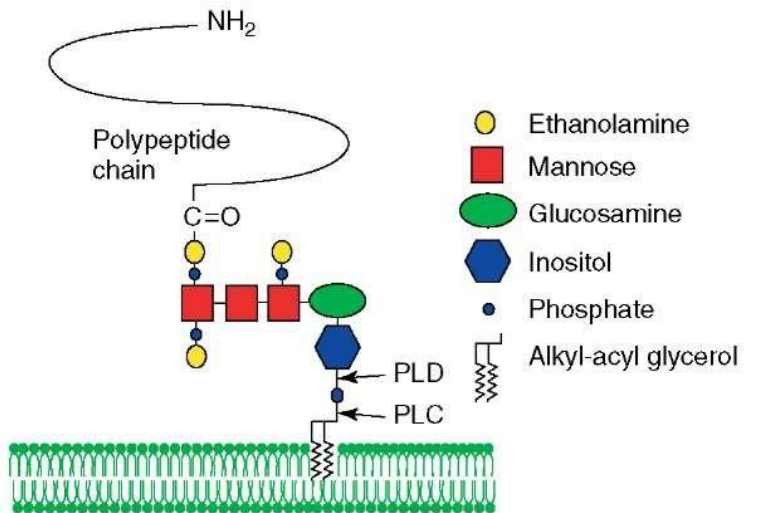
The sorting system depends on the cell type:

e.g: In cultured **polarized epithelial cells** infected with the influenza virus HA, progeny viruses bud only from the apical membrane, whereas in cells infected with vesicular stomatitis virus (VSV), progeny viruses bud only from the basolateral membrane.



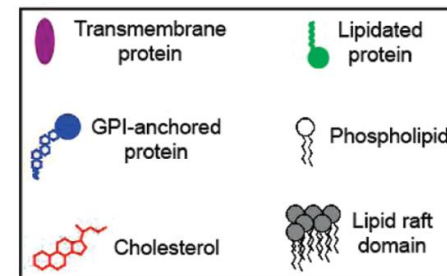
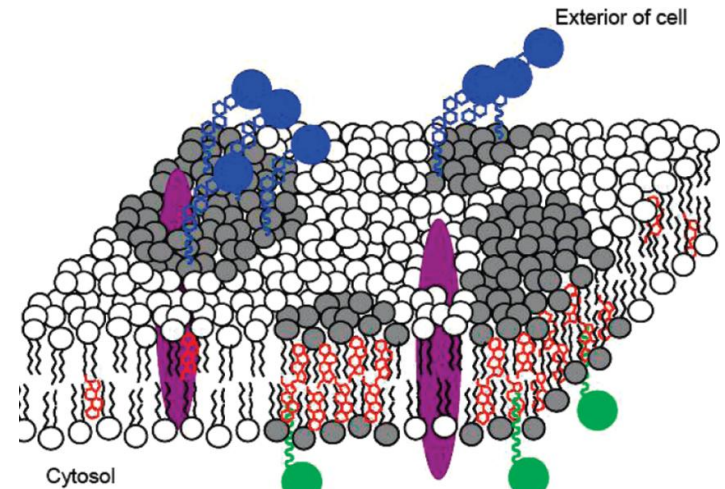
GPI-anchored proteins are targeted to the apical membrane of epithelial cells

In **polarized epithelial cells** GPI-anchored proteins are targeted to the apical membrane .
GPI = *glycosylphosphatidylinositol*



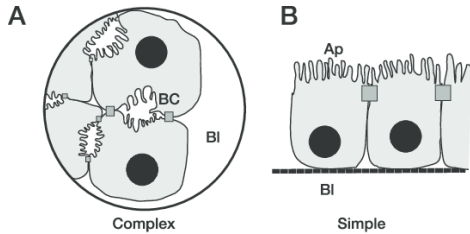
<http://what-when-how.com/proteomics/gpi-anchors-proteomics/>

In membranes GPI-anchored proteins are clustered into **lipid rafts**, which are microdomains rich in sphingolipids and cholesterol. Cleavage of the group by phospholipases (PLD, PLC) will result in controlled release of the protein from the membrane.



Biochemistry 2008, 47, 6991–7000

A different mechanism for sorting apical and basolateral proteins is present in hepatocytes



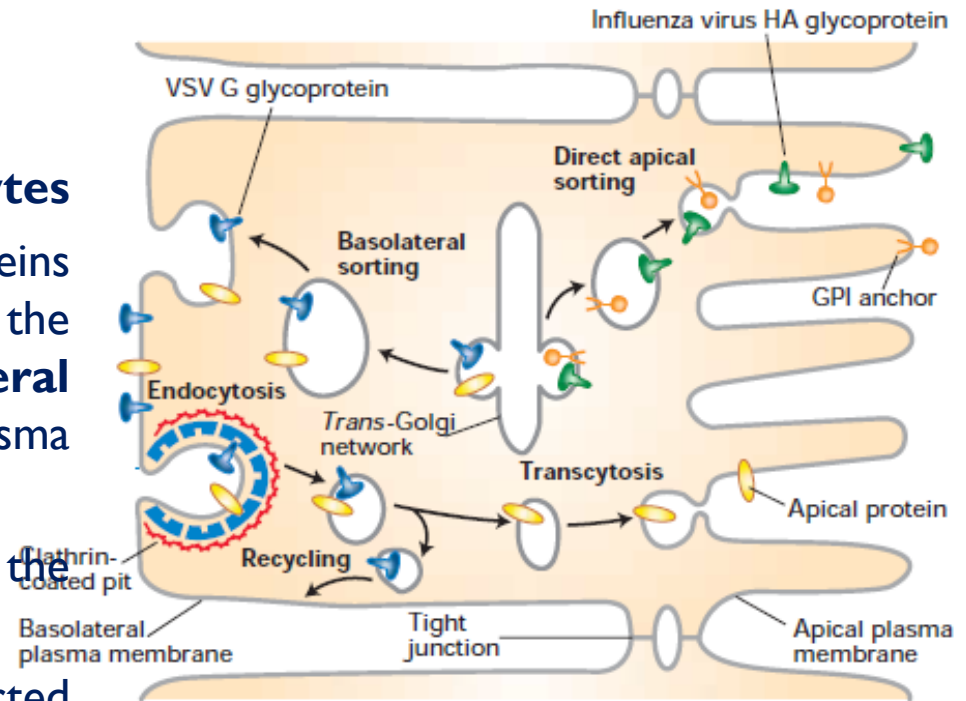
In hepatocytes

newly made apical and basolateral proteins are both transported in vesicles from the *trans-Golgi* network to **the basolateral region** and incorporated into the plasma membrane by exocytosis

Here all proteins are endocytosed in the same vesicles, but:

✓ the **basolateral proteins** are sorted into transport vesicles that recycle them to the basolateral membrane.

✓ the **apically destined endocytosed proteins** are sorted into transport vesicles that move across the cell and fuse with the apical membrane, a process called **transcytosis**.



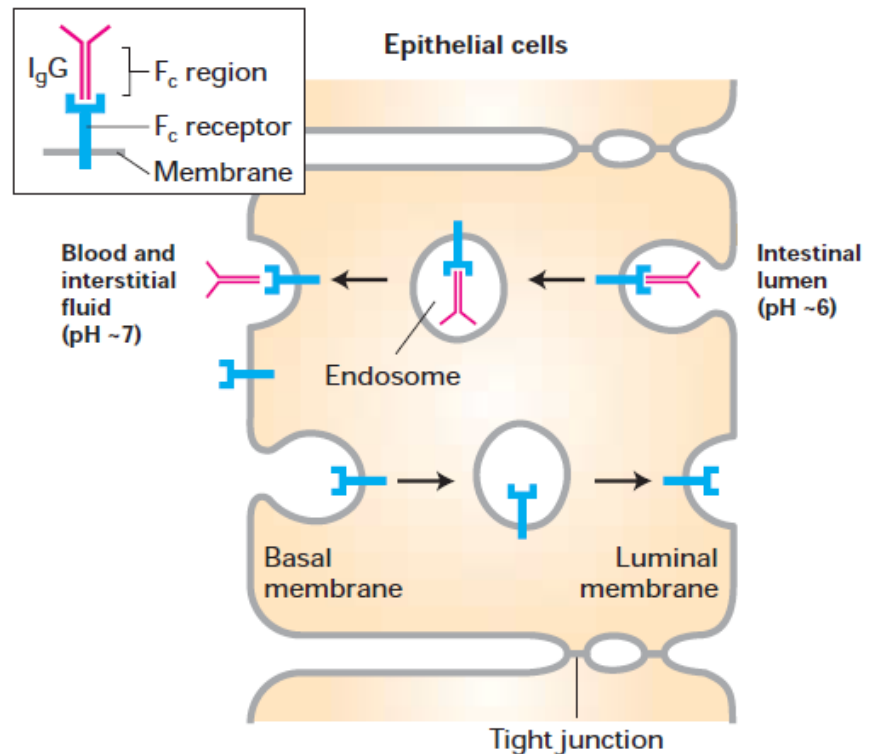
Transcytosis Moves Some Endocytosed Ligands Across an Epithelial Cell Layer

Transcellular transport, which **combines endocytosis and exocytosis**, also can be employed to **import an extracellular ligand** from one side of a cell and secrete it from the plasma membrane at the opposite side.

Transcytosis occurs mainly in sheets of polarized epithelial cells.

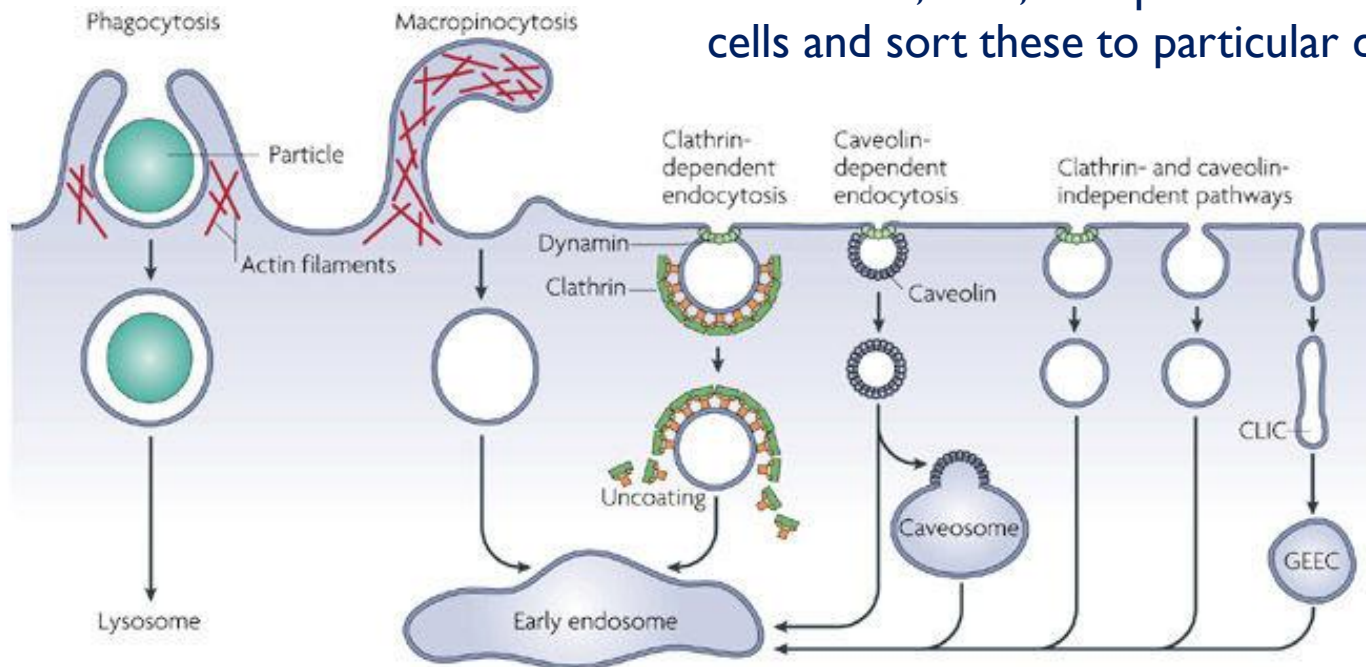
Maternal immunoglobulins (antibodies) contained in ingested breast milk are transported across the intestinal epithelial cells of the newborn mouse and human by transcytosis.

The Fc receptor mediates this movement by pH-dependent binding to Ig.



Pathways of entry into cells: Endocytosis

All cells take up macromolecules, particulate substances, and, in specialized cases, even other cells and sort these to particular destinations.

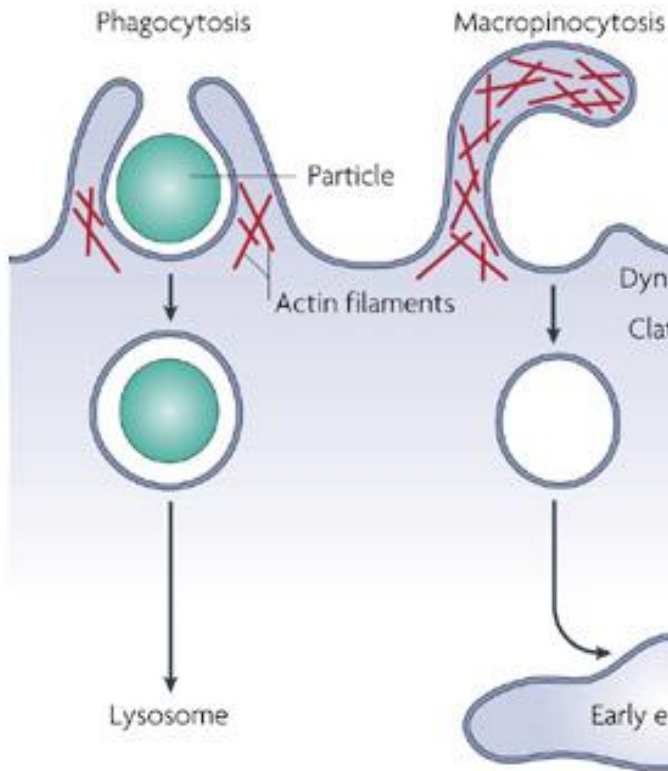


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Several **endocytic pathways** are shown in a small section of plasma membrane; they include phagocytosis, macropinocytosis, clathrin-dependent endocytosis, caveolin-dependent endocytosis, and three clathrin- and caveolin-independent pathways.



Actin-dependent endocytosis



Phagocytosis, is a regulated nonselective actin-mediated process in which plasma membrane protrusions on the right and left sides of the particle envelop and engulf the particle (phagosomes). The protrusions are curved around the particle and contain crisscrossed red lines that represent **actin filaments**.

Macropinocytosis is a regulated form of endocytosis that mediates the **non-selective** uptake of solute molecules, nutrients and antigens. A membrane protrusion that resembles a wave is filled with **actin filaments**.

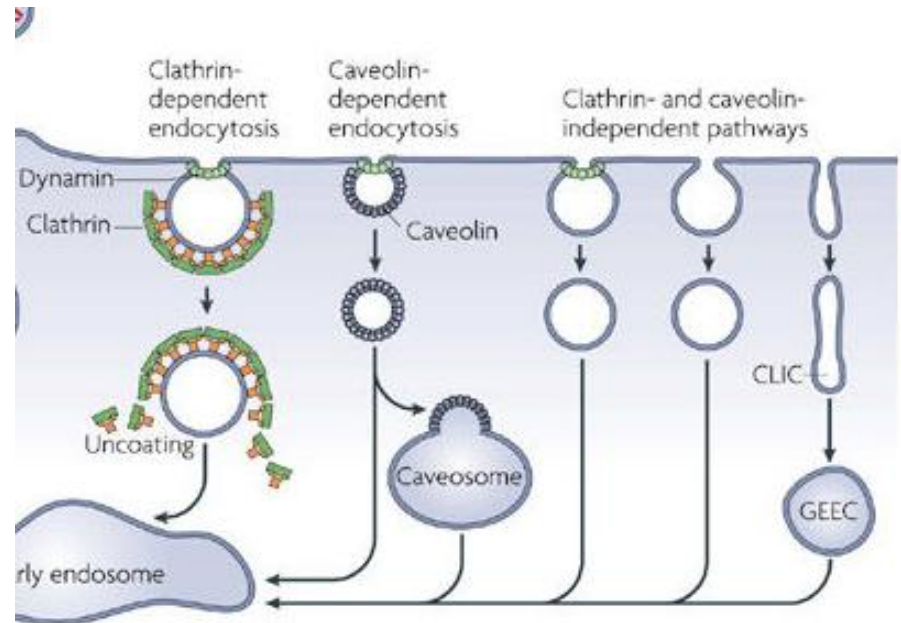
A few cell types (e.g., macrophages) can take up whole bacteria and other large particles

Other endocytic pathways

All eukaryotic cells continually engage in endocytosis: a process in which a small region of the plasma membrane invaginates to form a membrane-limited vesicle about 50-100 nm in diameter (pinocytotic vesicles).

Clathrin-dependent endocytosis or **Receptor-mediated endocytosis**: specific receptors on the cell surface binds tightly to an extracellular macromolecular ligand that it recognizes and the complex is internalized

caveolin-dependent endocytosis: starting from invaginations of the plasma membrane (caveolae)

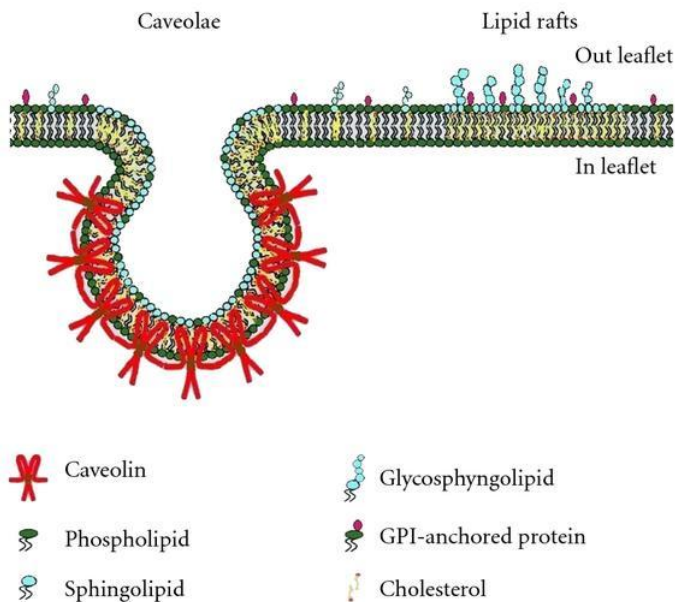


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Three other forming vesicles pathways independent from clathrin and caveolin have been described.

Caveolin-dependent endocytosis

A different, less well-understood mechanisms by which cells can form pinocytic vesicles initiates at **caveolae** (“little cavities”), which are present in the plasma membrane of most cell types, and in some of these they are seen as deeply invaginated flasks (approx. 50 nm in diameter).



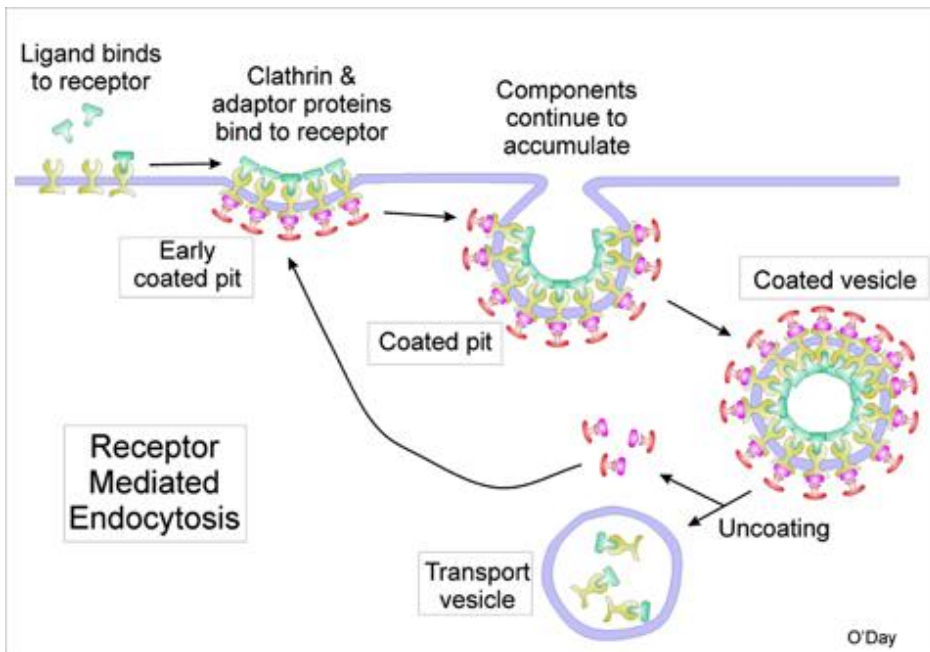
Caveolae are thought to invaginate and collect cargo proteins by virtue of the **lipid composition** of the caveolar membrane.

Caveolae form from **lipid rafts**, especially rich in cholesterol, glycosphingolipids, and GPI-anchored membrane proteins. The major structural protein in caveolae is **caveolin**, a multipass integral membrane protein.

Other possible functions: regulation of signalling and lipid homeostasis.

Receptor-Mediated Endocytosis and Sorting of Internalized Proteins

Receptor-mediated endocytosis occurs via **clathrin/AP2**-coated pits and vesicles in a process similar to the packaging of lysosomal enzymes by mannose 6-phosphate (M6P) in the *trans-Golgi network*

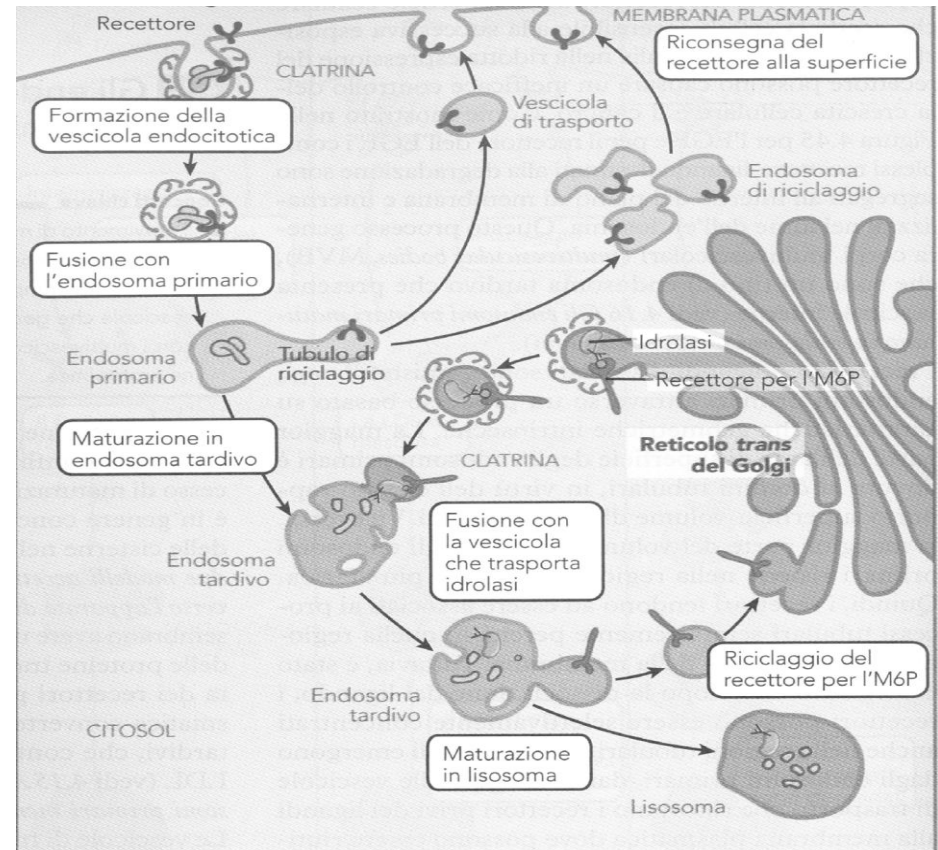


Many internalized ligands have been observed in these pits and vesicles, which are thought to function as intermediates in the endocytosis of most ligands bound to cell-surface receptors. **Some receptors are clustered** over clathrin-coated pits even in the absence of ligand. **Other receptors diffuse freely** in the plane of the plasma membrane but undergo a conformational change when binding to ligand, so that when the receptor-ligand complex diffuses into a clathrin-coated pit, it is retained there.

The endocytic pathway from the plasma membrane to lysosomes

The **endocytic pathway** of mammalian cells consists of distinct membrane vesicles, which internalize molecules from the plasma membrane and:

- 1) recycle them to the surface (as in **early endosomes** and **recycling endosomes**), or
- 2) sort them to degradation (as in **late endosomes** and **lysosomes**).



Most of the plasma membrane components (proteins and lipid) that are endocytosed are continually returned to the cell surface by exocytosis. (up to 50% / hr).

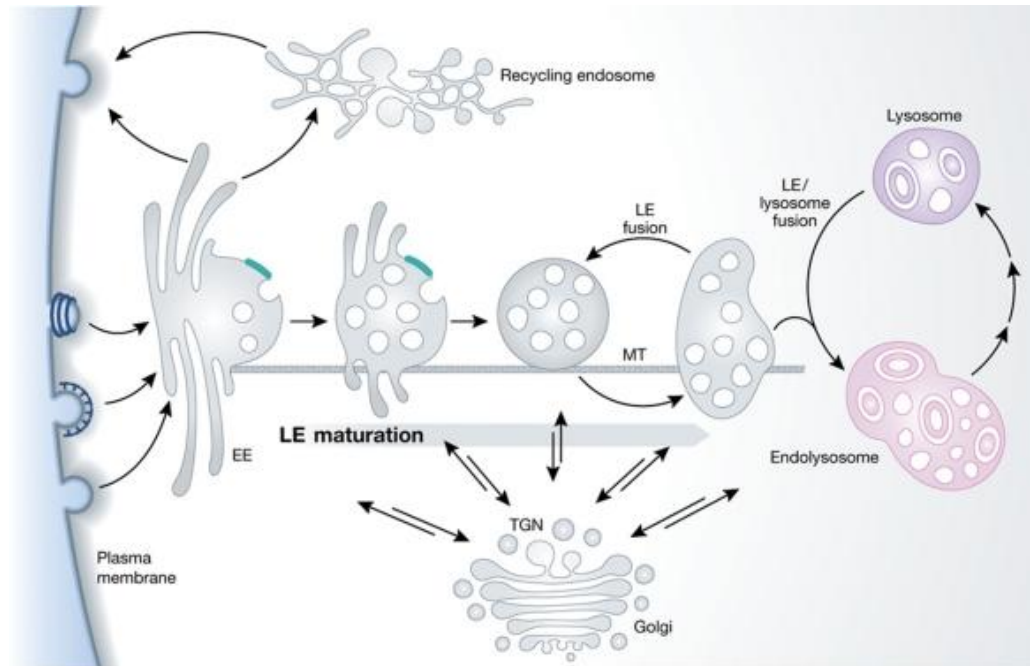
Maturation of early endosome

Maturation from **early endosomes (EE)** to **late endosomes (LE)**.

EEs accumulate cargo and support recycling to the plasma membrane.

LEs carry a selected subset of endocytosed cargo from the EE, and move towards the perinuclear space along microtubules (MT),

LEs eventually turn into **multivesicular bodies**, which contain invaginated membrane and internal vesicles (ILV).

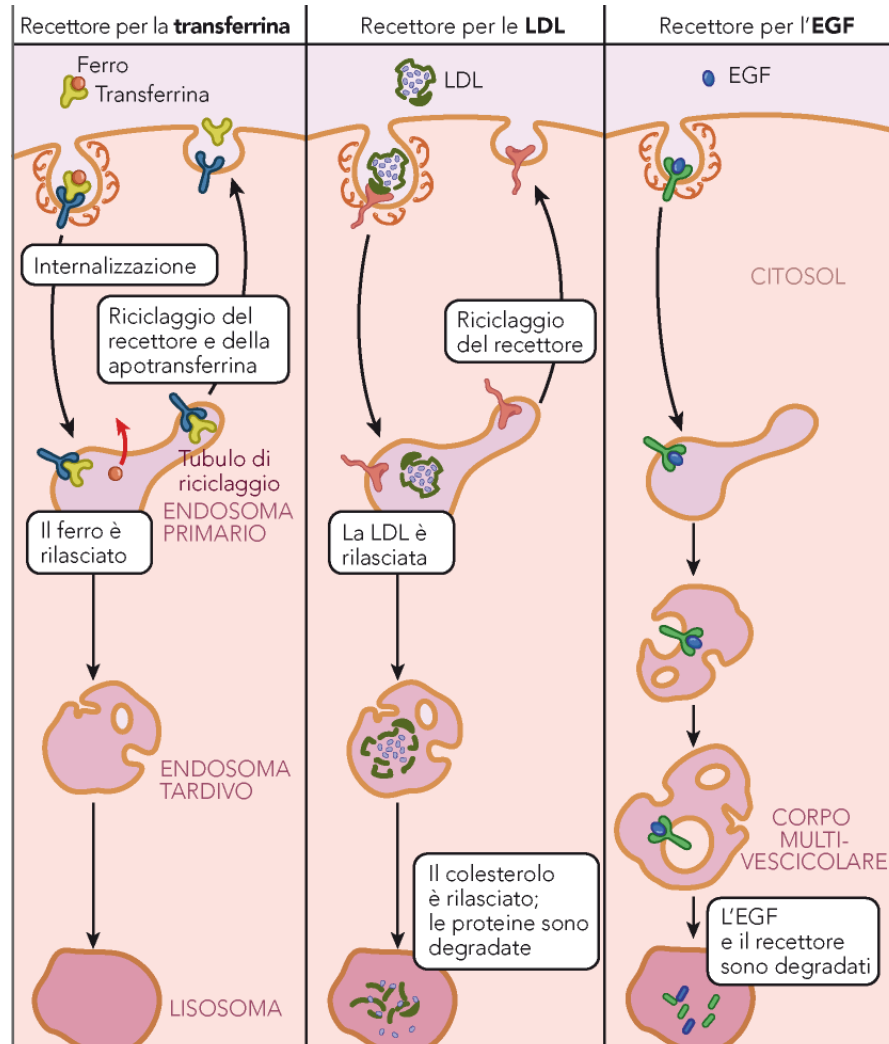


EMBO J. 2011 Aug 31; 30(17): 3481–3500.

The role of LEs as feeder system is to deliver this mixture of endocytic and secretory components to lysosomes. LEs communicate with the TGN via transport vesicles. The fusion of an endosome with a lysosome generates a transient hybrid organelle, the endolysosome, in which active degradation takes place.

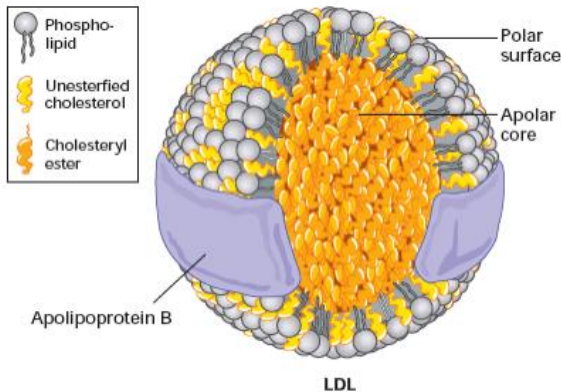


Cell Internalized receptors may be recycled or degraded



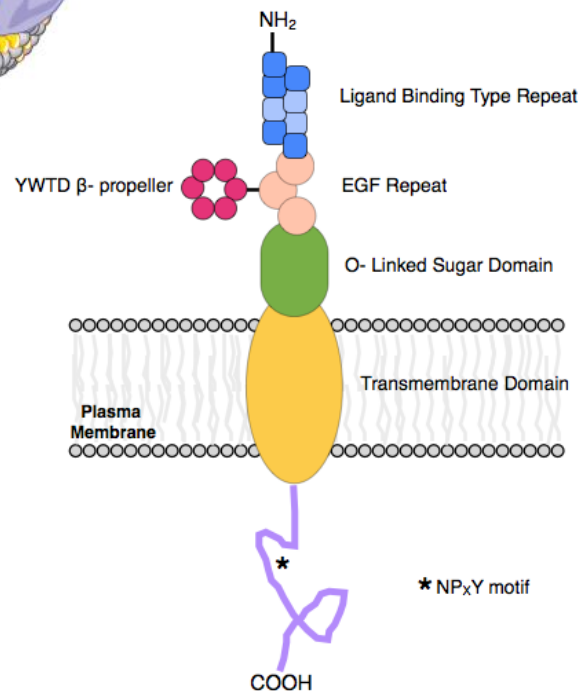
Receptors for LDL Contain Sorting Signals That Target Them for Endocytosis

Low density lipoprotein (LDL) is one of several complexes that carry cholesterol through the bloodstream



LDL particle, a sphere 20–25 nm, outer phospholipid shell, protein apoB-100 and cholesteryl esters.

Most mammalian cells produce cell-surface receptors that specifically bind to **apoB-100** and internalize LDL particles by receptor-mediated endocytosis.

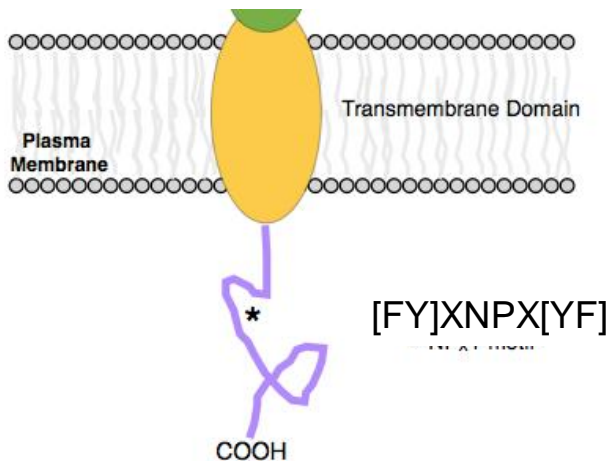


An 839-residue glycoprotein with a single transmembrane segment; short C-terminal cytosolic segment and long N terminal exoplasmic segment that contains a **β-propeller domain** and a **ligand-binding domain (arm)**.

7 cysteine-rich imperfect repeats form the ligand-binding domain, which interacts with the apoB-100 molecule in a LDL particle.

Mutation in the LDL receptor impairs endocytosis

Studies of the inherited disorder *familial hypercholesterolemia* (high serum levels of cholesterol) led to discovery of the LDL receptor and the initial understanding of the endocytic pathway.



Mutant receptors bind LDL normally, but the LDL-receptor complex cannot be internalized by the cell and is distributed over the cell surface rather than being confined to clathrin/AP2-coated pits.

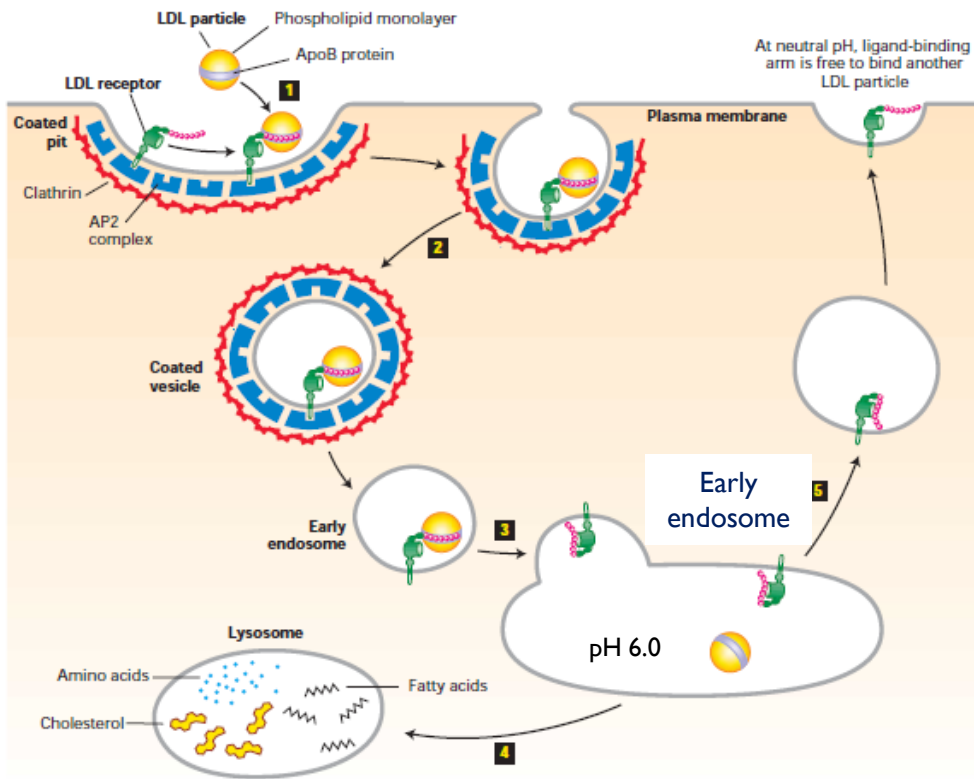
Four-residue motif in the cytosolic segment of the receptor is crucial for its internalization **Asn-Pro-X-Tyr** ([FY]XNPX[YF]) because it is recognized by the AP2 complex.

A mutation in any of the conserved residues of the NPXY signal will abolish the ability of the LDL receptor to be incorporated into coated pits.

The gene encoding the AP2 subunit protein that binds the NPXY sorting signal may be defective giving a similar phenotype.



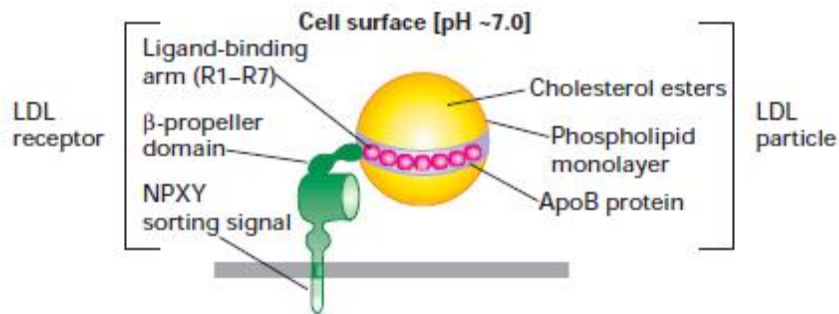
The Acidic pH of Late Endosomes Causes Most Receptor-Ligand Complexes to Dissociate



Internalized receptor-ligand complexes commonly follow the pathway depicted for the M6P receptor in Figure . Cell-surface receptors that undergo endocytosis will repeatedly deposit their ligands within the cell and then **recycle** to the plasma membrane (10-20 min).

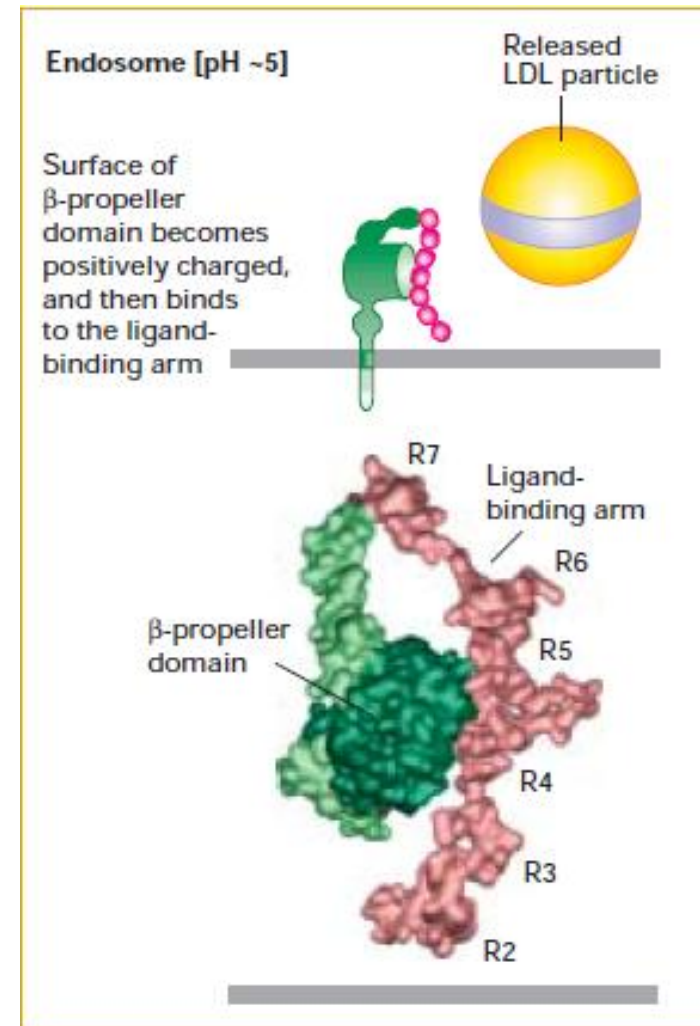
Receptors typically dissociate from their ligands within **early endosomes**, the first vesicle encountered by receptor-ligand complexes whose luminal pH is sufficiently acidic to promote dissociation of most endocytosed receptors from their tightly bound ligands.

The mechanism by which the LDL receptor releases bound LDL particles



At the endosomal pH of 5.5–6.0, **histidine residues** in the **β -propeller domain** of the receptor become protonated, forming a site that can bind with high affinity to the negatively charged repeats in the ligand-binding domain.

This intramolecular interaction **sequesters the repeats** in a conformation that cannot simultaneously bind to apoB-100, thus causing release of the bound LDL particle.

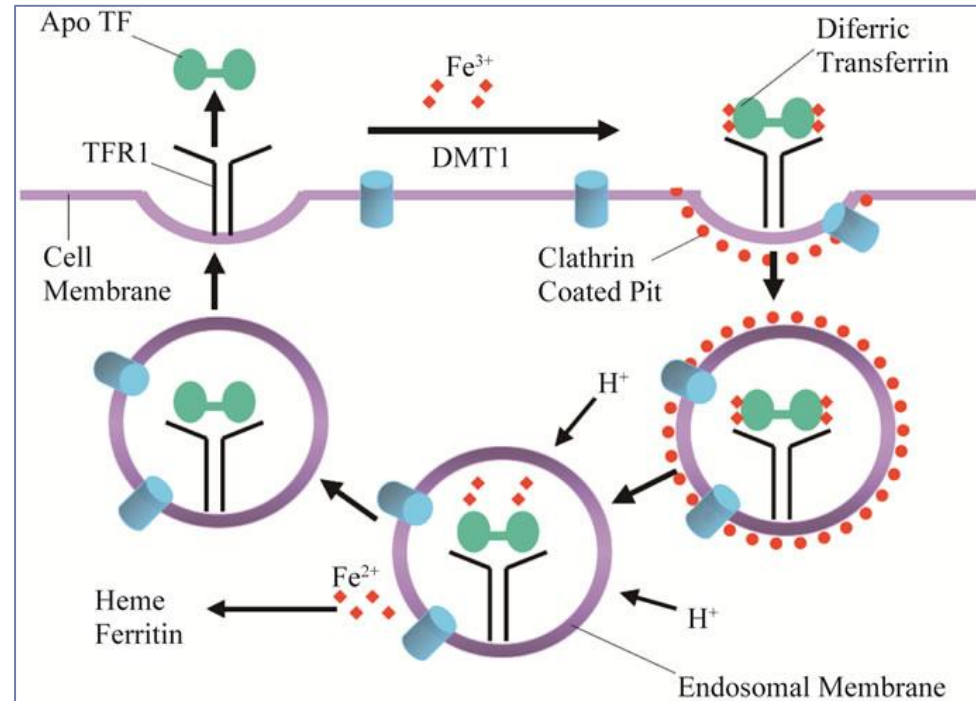


The Endocytic Pathway Delivers Iron to Cells Without Dissociation of Receptor-Transferrin Complex in Endosomes

Transferrin (blood glycoprotein) transports iron to all tissue cells from the liver and intestine. The iron-free form, *apotransferrin*, binds two Fe^{3+} ions very tightly to form *ferrotransferrin*. All mammalian cells contain cell-surface **Fe-transferrin receptors** (TFR1) that bind it at neutral pH. after which the receptor- bound ferrotransferrin is subjected to endocytosis

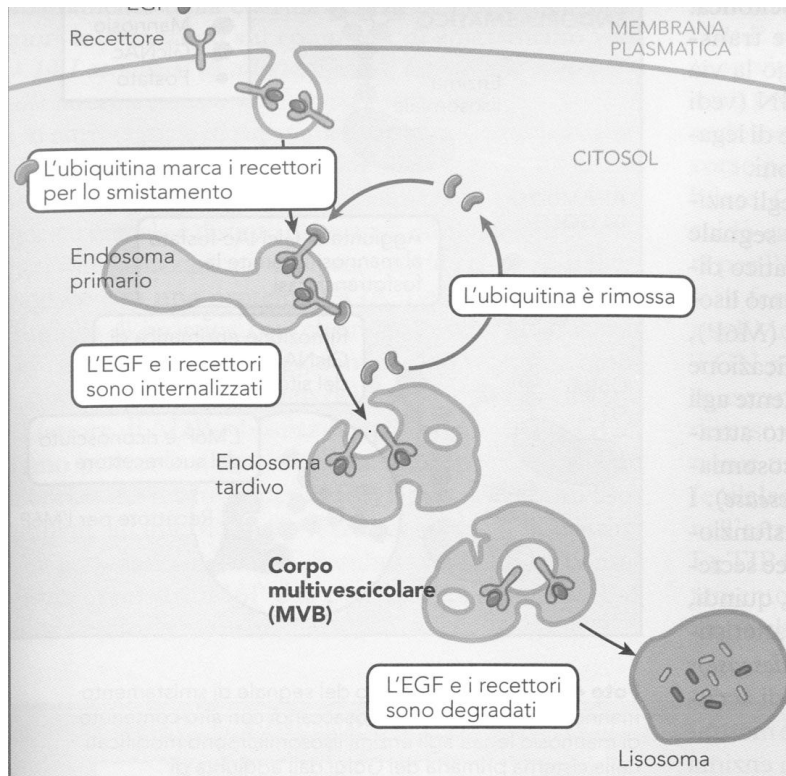
At a pH below 6.0, the bound Fe^{3+} dissociate from *ferrotransferrin*, but the *apotransferrin* **does not dissociate** from the receptor and is secreted from the cell within minutes after being endocytosed.

Apotransferrin binds to its receptor at a pH of 5.0-6.0 and dissociates from the its receptor when the recycling vesicles fuse with the plasma membrane (pH=7)



EGFR signaling may be attenuation by degradation

The sensitivity of a cell to a particular signaling molecule can be down-regulated by **endocytosis of its receptors**, thus decreasing the number on the cell surface (*desensitization of receptors*).

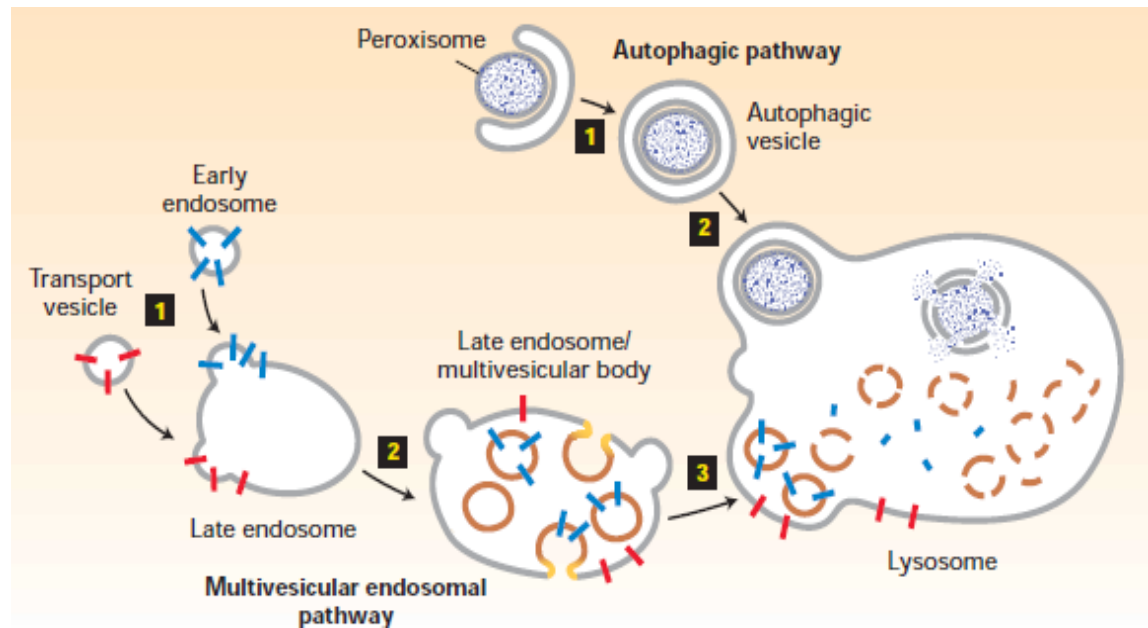


Clathrin-mediated endocytosis is the major pathway of **epidermal growth factor receptor (EGFR)** internalization. (and other tyrosine kinase receptors, i.e. insulin receptor). It is commonly believed that this pathway mediates **long-term attenuation** of EGFR signaling by targeting the receptor for degradation. Cytosolic **ubiquitin** marks receptors that have to be included in the ILVs (intra luminal vesicles) of the multivesicular body (MVB). The endosomal sorting complex required for transport (ESCRT), machinery is responsible for sorting of ubiquitinated membrane proteins into ILVs

Specialized Vesicles Deliver Cell Components to the Lysosome for Degradation

Endocytosed receptor proteins targeted to the lysosomes were primarily associated with membrane fragments and small vesicles within the interior of the late endosome (ILVs) rather than with the surface membrane.

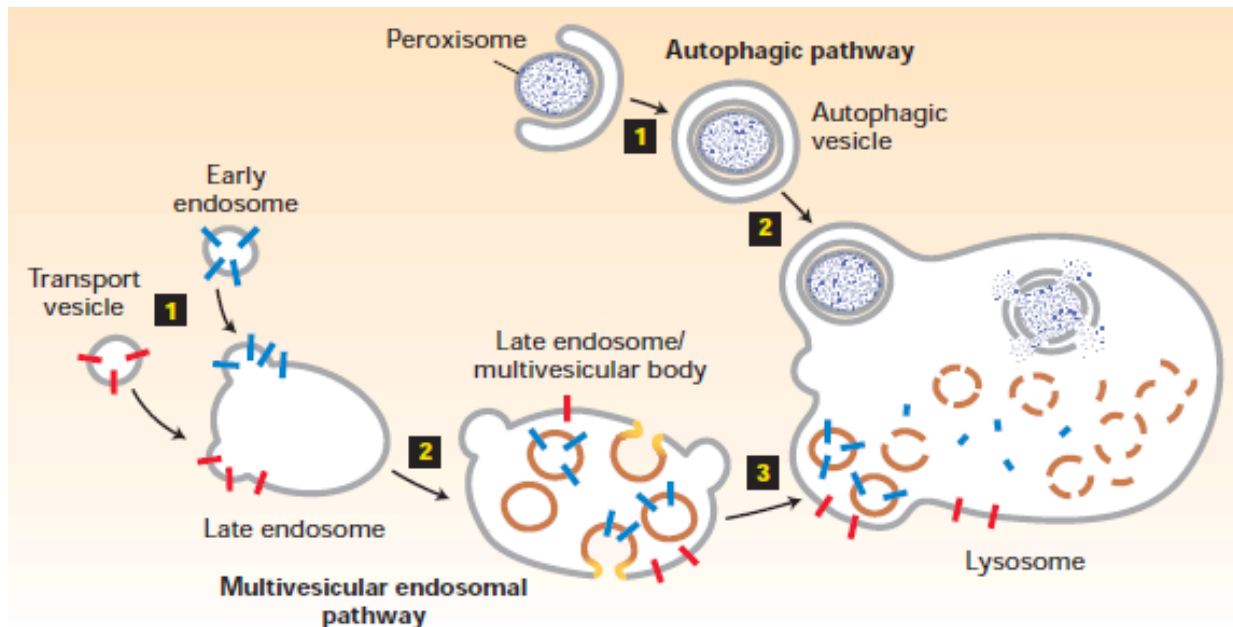
Although ILVs are similar in size and appearance to transport vesicles, they differ topologically. Transport vesicles bud outward from the surface of a donor organelle into the cytosol, whereas vesicles within the endosome bud inward from the surface into the lumen



The sorting of proteins in the endosomal membrane (surface membrane or ILVs) determines which ones will remain on the lysosome surface (e.g., pumps and transporters) and which ones will be incorporated into internal vesicles and ultimately degraded in lysosomes.

Autophagic Vesicles: the delivery of bulk amounts of cytosol

The delivery of bulk amounts of cytosol or entire organelles to lysosomes and their subsequent degradation is known as **autophagy** (“eating oneself”).



Autophagic vesicle envelops a region of the cytosol or an entire organelle (e.g., peroxisome, mitochondrion).

The outer membrane of an autophagic vesicle can fuse with the lysosome delivering a large vesicle, bounded by a single membrane bilayer, to the interior of the lysosome.