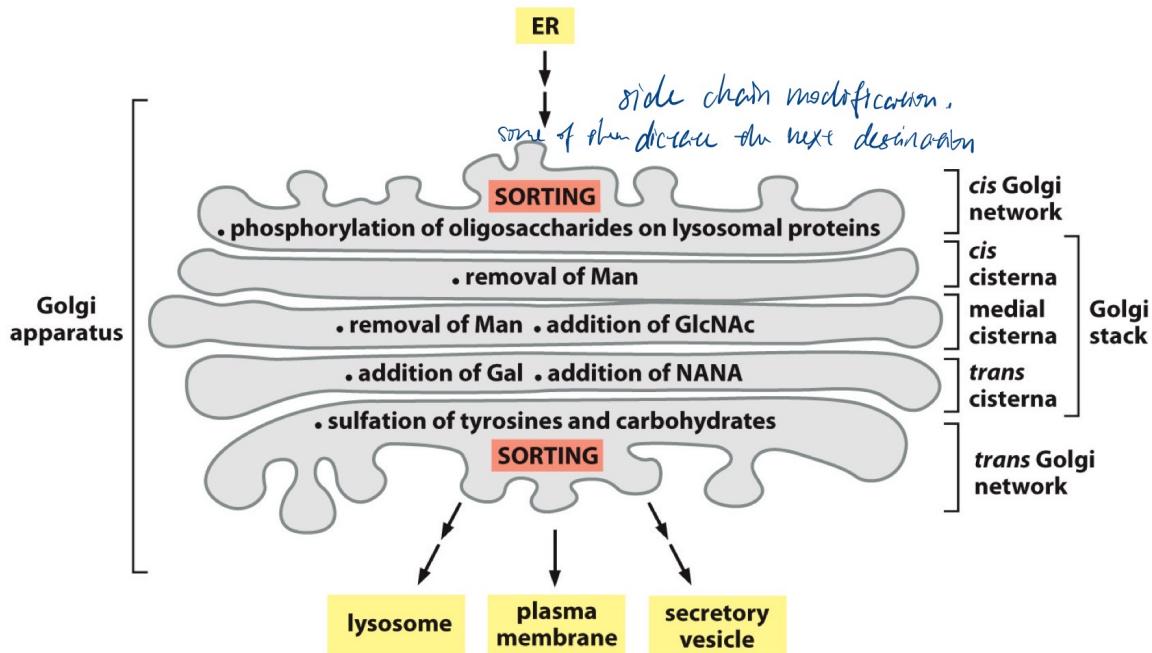


The CGN is a collection of **fused vesicular tubular clusters arriving from the ER**. Proteins and lipids enter the cis Golgi network and exit from the trans Golgi network, bound for the cell surface or another compartment. Both networks are important for protein sorting: proteins entering the CGN can either move onward in the Golgi apparatus or be returned to the ER. Similarly, proteins exiting from the TGN move onward and are sorted according to their next destination: **endosomes, secretory vesicles, or the cell surface**. They also can be returned to an earlier compartment. Some membrane proteins are retained in the part of the Golgi apparatus where they function.

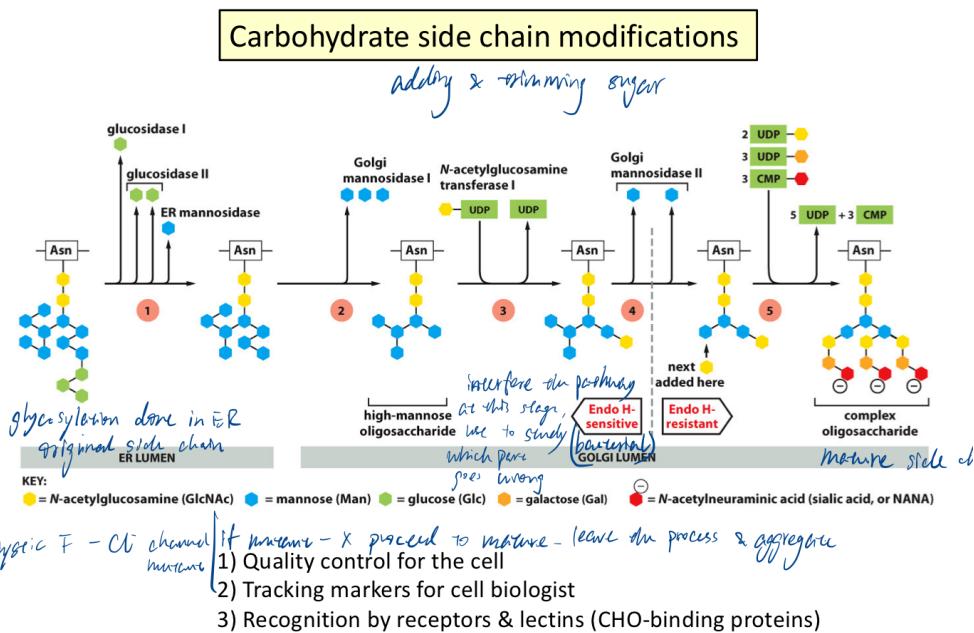
- **One important modification in Golgi is the glycosylation**

- ▼ The steps to complete glycosylation in Golgi



- The function and localisation of each processing step is determined by:
 - biochemical subfractionation of the Golgi
 - EM after staining with Ab specific for some processing enzymes
 - The localisation of enzymes are not restricted to a particular cisterna — graded distribution across the stack
 - **All the resident Golgi proteins are membrane-bound!**
- Man: Mannose
- GlcNac: N-acetylglucosamine
- Gal: galactose
- NANA: N-acetylneurameric acid (sialic acid)

mis folding protein disease



1. Processing begins in the **ER** with the **removal of the glucoses** from the oligosaccharide initially transferred to the protein. Then a **mannosidase** in the ER membrane **removes a specific mannose**. The remaining steps occur in the Golgi stack.
2. Golgi mannosidase I removes three more **mannoses — high-mannose oligosaccharide**.
3. N-acetylglucosamine transferase I then **adds an N-acetylglucosamine**.
4. Golgi mannosidase II then removes two additional mannoses. This yields the final core of three mannoses that is present in a complex oligosaccharide. At this stage, the **bond between the two N-acetylglucosamines in the core becomes resistant to attack by a highly specific endoglycosidase (Endo H)**.

Because all later structures in the pathway are also Endo H-resistant, treatment with this enzyme is widely used to distinguish complex from high-mannose oligosaccharides.
5. Additional N-acetylglucosamines, **galactoses, and sialic acids** are added. These final steps in the synthesis of a complex oligosaccharide occur in the cisternal compartments of the Golgi apparatus: three types of glycosyl

transferase enzymes act sequentially, using sugar substrates that have been activated by linkage to the indicated nucleotide; the membranes of the Golgi cisternae contain specific carrier proteins that allow each sugar nucleotide to enter in exchange for the nucleoside phosphates that are released after the sugar is attached to the protein on the luminal face.

▼ Variations of Golgi modification:

- Human genome encodes various Golgi glycosyl transferases and glycosidases to glycosylate the proteins and lipids differently in different cell types and at varying times.

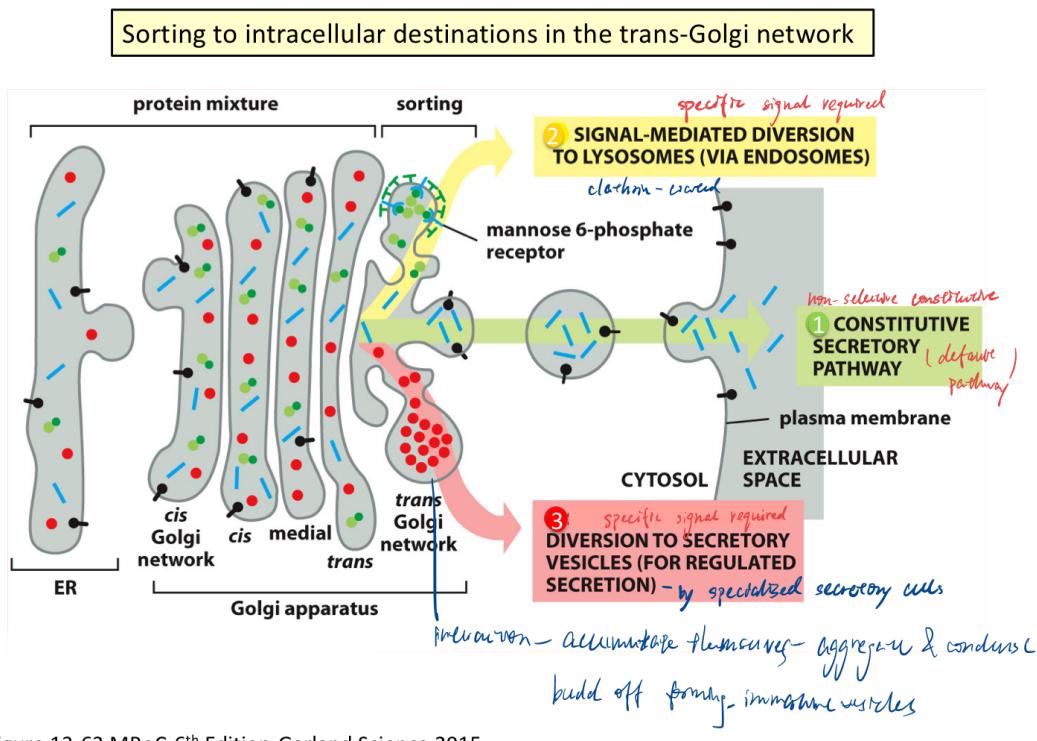
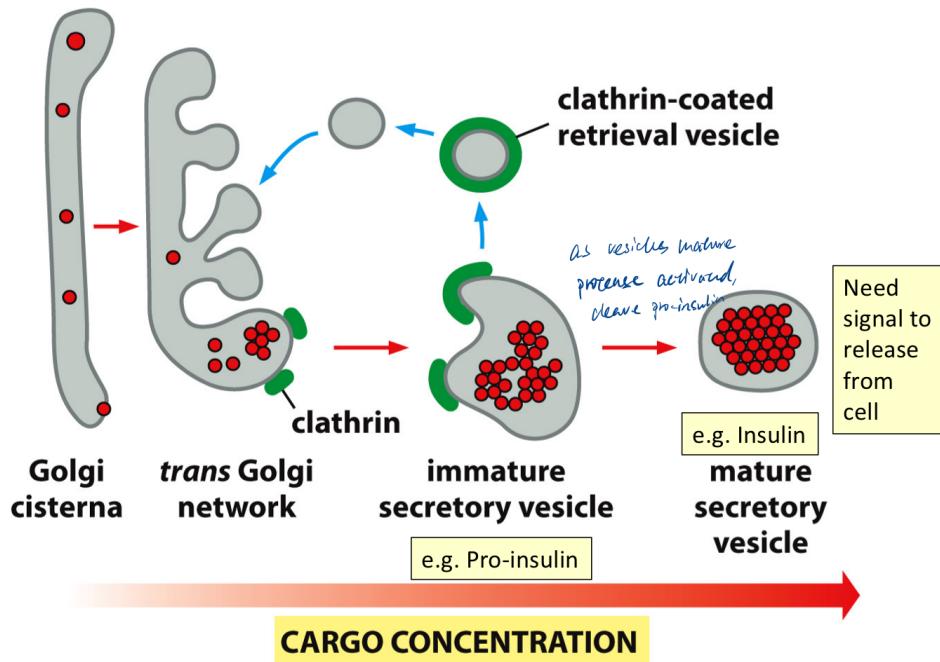


Figure 12.22 MDU 6th Edition, Chapter 12, © 2015

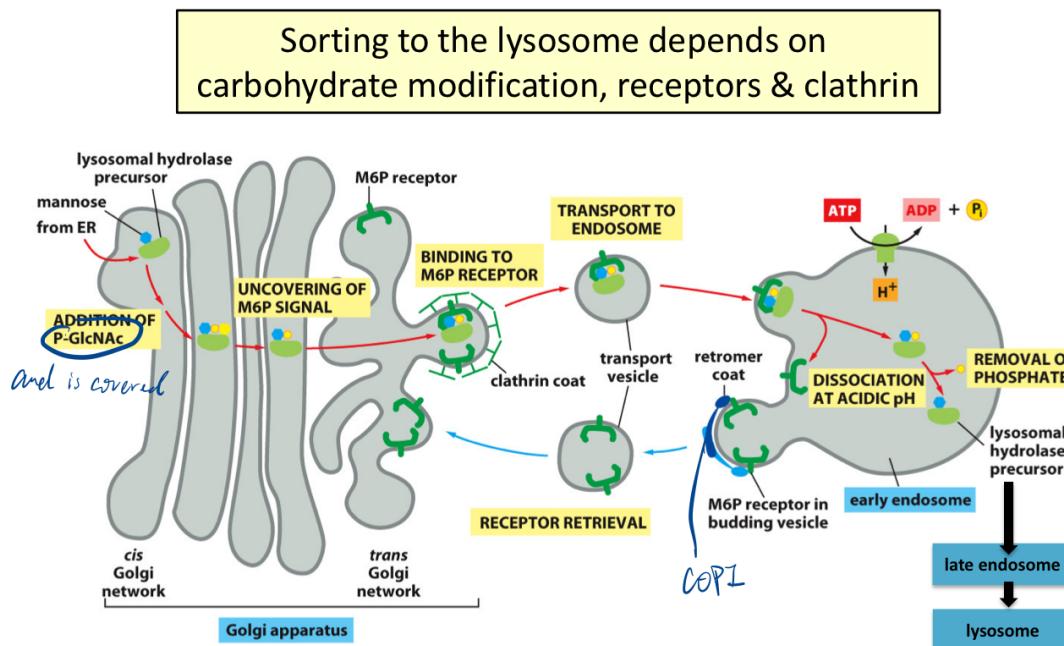
▼ Secretory vesicle exocytosis pathway — aggregation of the secretory proteins



- selective aggregation of the secretory proteins
- Clumps of aggregated, electron-dense material can be detected by electron microscopy in the lumen of the TGN (trans-Golgi network). **The signals** that direct secretory proteins into such aggregates are not well defined and may be quite diverse. When a gene encoding a secretory protein is artificially expressed in a secretory cell that normally does not make the protein, the foreign protein is appropriately packaged into secretory vesicles. This observation shows that, **although the proteins that an individual cell expresses and packages in secretory vesicles differ, they contain common sorting signals**, which function properly even when the proteins are expressed in cells that do not normally make them.
- Initially, the membrane of the secretory vesicles that leave the TGN is only loosely wrapped around the clusters of aggregated secretory proteins. Morphologically, these immature secretory vesicles resemble dilated trans Golgi cisternae that have pinched off from the Golgi stack. **As immature secretory vesicles mature, clathrin-coated transport vesicles bud from them and go back to the TGN** (Figure 13–42). This recycling process not

only **returns Golgi components to the Golgi apparatus**, but also **serves to concentrate the contents of secretory vesicles**.

▼ Selective packaging of **Lysosomal Hydrolases** to **Lysosome** - for mediating protein degradation



Defects in lysosomal enzyme targeting lead to lysosomal storage diseases (swollen and clogged up lysosomes)

defects in P-GlcNAc enzyme, m6p R → lysosomal enzymes X transported to lysosomes - X digest proteins

- Adding P-GlcNAc
- **Uncovering Lysosomal hydrolases marker: M6P signal**
- M6P binds to Transmembrane **M6P receptor proteins** present in **TGN**
- M6P dissociates from M6PR at endosome, where the pH is lower
- M6PR are retrieved from endosome to Golgi

Symptoms of lysosomal storage diseases

Delay in intellectual development (neuronal *recycling*)
defects) and physical development (endocytosis)

Seizures (neuronal defects)

Facial and other bone deformities (bone defects)
Joint stiffness and pain

Difficulty breathing

Problems with vision and hearing

Anemia, nosebleeds, and easy bleeding or bruising

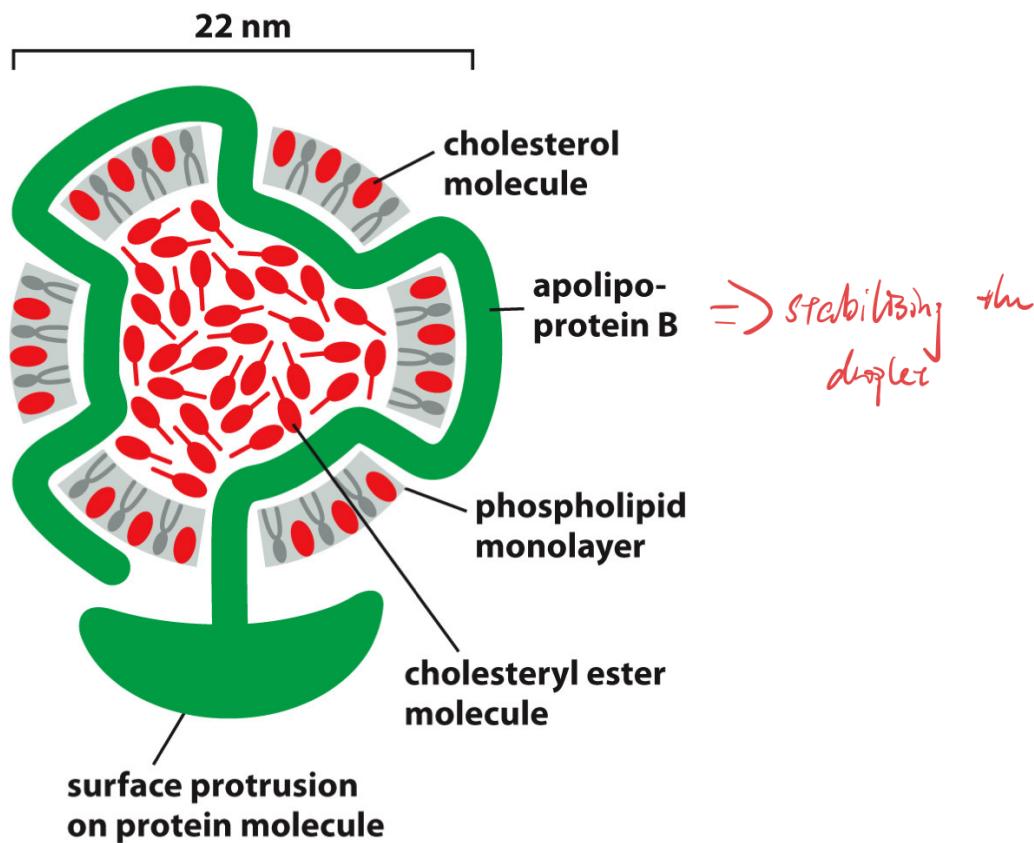
Swollen abdomen due to enlarged spleen or liver

- Mutated lysosomal hydrolases / missorting because of the defective Golgi resident membranes — number of human lysosomal storage diseases - symptoms often associated with NS
 - pathologically resulted from accumulation of undigested substrate in lysosomes — consequently form large inclusions in the cell

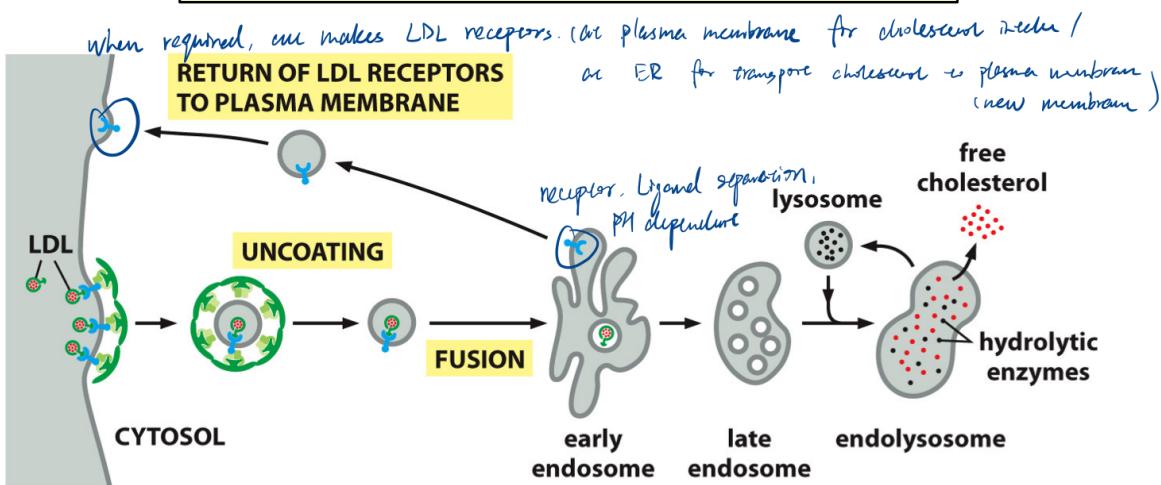
▼ Defective receptor-mediated endocytosis & hypercholesterolaemia

- the uptake cholesterol is required to make new membrane
- if the cholesterol cannot be taken up effectively, the accumulated cholesterol in blood can lead to :
 - atherosclerotic plaque formation in the blood vessels

- lipid and fibrous tissue deposition → block arterial blood flow → stroke & heart attack
- ▼ most cholesterol in the blood exists as low-density lipoproteins (LDL):



Cholesterol (LDL) uptake is clathrin-dependent



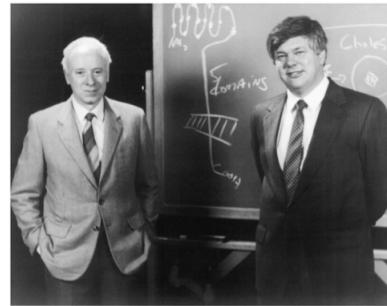
- Receptor-mediated endocytosis of LDL (cholesterol)
- Signal: -FDNPVY-

After shedding their clathrin coats, the vesicles deliver their contents to early endosomes. Once the LDL and LDL receptors encounter the low pH in early endosomes, LDL is released from its receptor and is delivered via late endosomes to lysosomes. There, the cholesterol esters in the LDL particles are hydrolyzed to free cholesterol, which is now available to the cell for new membrane synthesis. If too much free cholesterol accumulates in a cell, the cell simultaneously shuts off endogenous cholesterol synthesis (Figure 12–64) and reduces exogenous cholesterol intake by shutting off the synthesis of LDL receptors.

- ▼ The LDL-mediated internalisation is found signalling sequence dependent
 - Why?
 - found that although the presence of LDL & still binds to the mutant receptor BUT the signalling sequence of LDLR change → no internalisation

How was the LDL receptor endocytosis signal discovered?

- Joseph Goldstein and Michael Brown were studying patients with familial hypercholesterolaemia

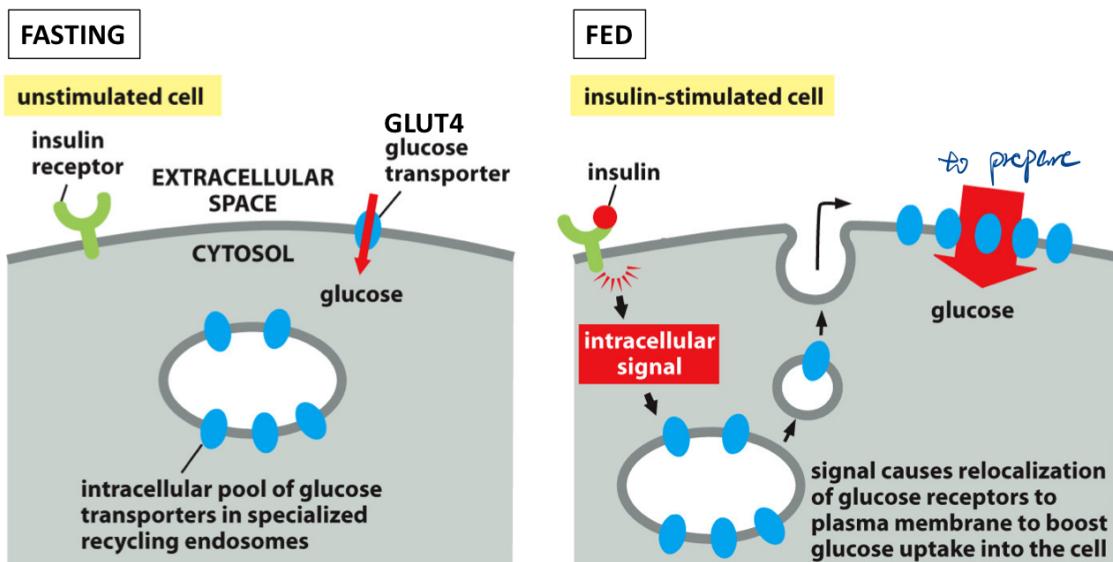


1985 Nobel Prize

- One patient had an amino acid change -NPVY- to -NPVC- in the cytoplasmic domain of the LDL receptor
 - LDL still binds the mutant receptor but fails to internalise
no endocytosis
 - LDL can not be cleared from the blood

Considered the first functional demonstration of receptor-mediated endocytosis

Insulin-mediated glucose uptake also depends on membrane traffic

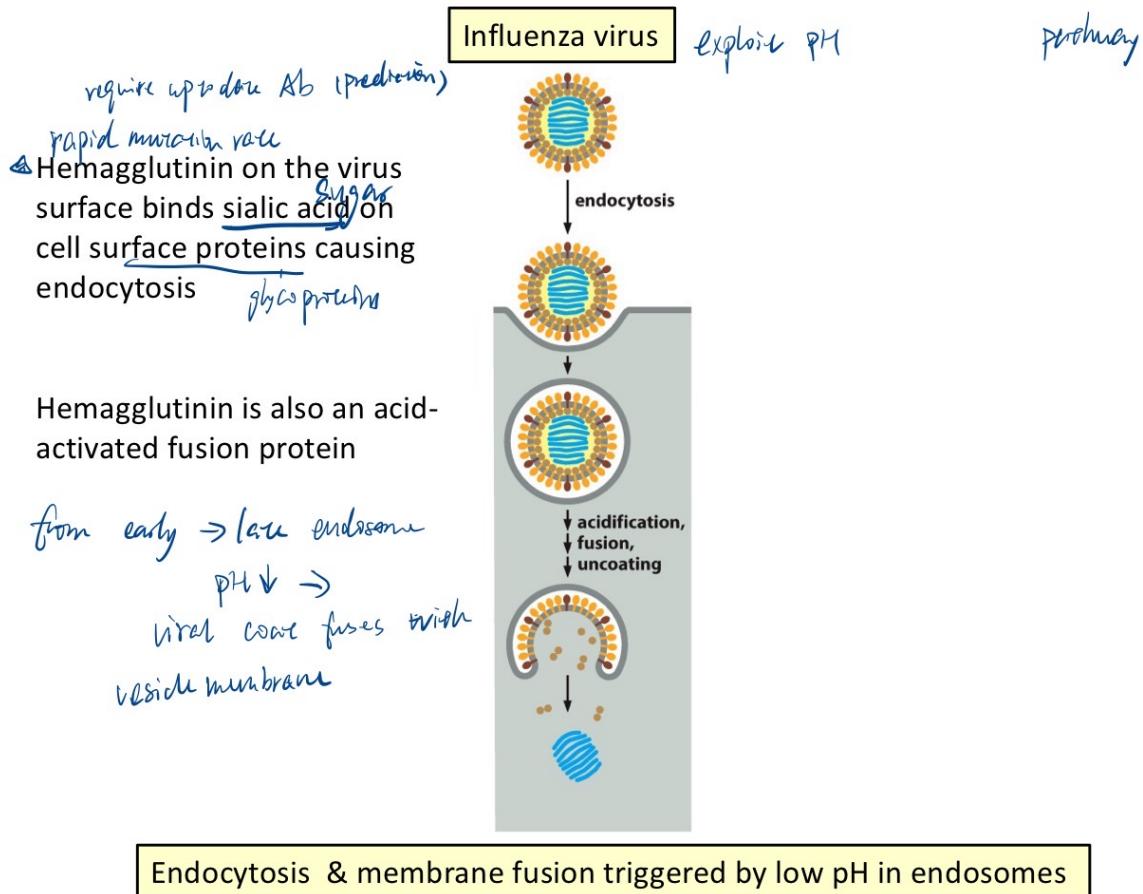


Type 1 diabetes-no insulin is made (autoimmune attack on pancreas) \rightarrow \times import glucose

special clathrin required for recycling endosomes transport GLUT4

Type 2 diabetes-insulin signaling defect (insulin resistance) failure to stimulate GLUT4 membrane traffic *Why is obesity obstruct the signal*

Figure 13-59 MBoC-6th Edition-Garland Science 2015



▼ Formation of intraluminal vesicles

- at endosome lumen — such maturing endosomes are also called multivesicular bodies

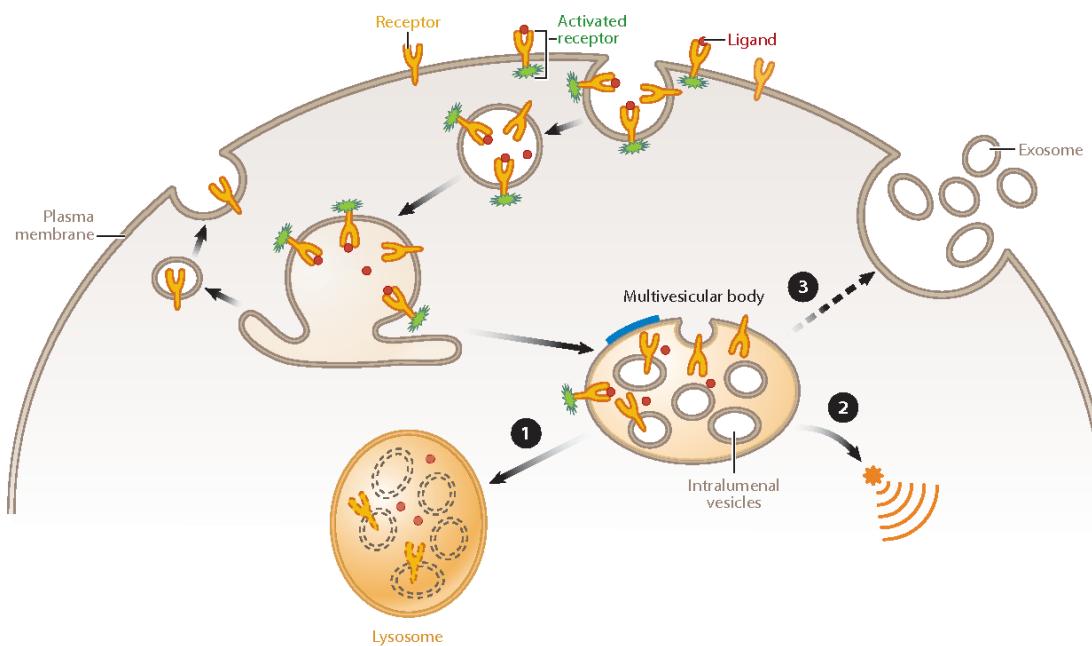
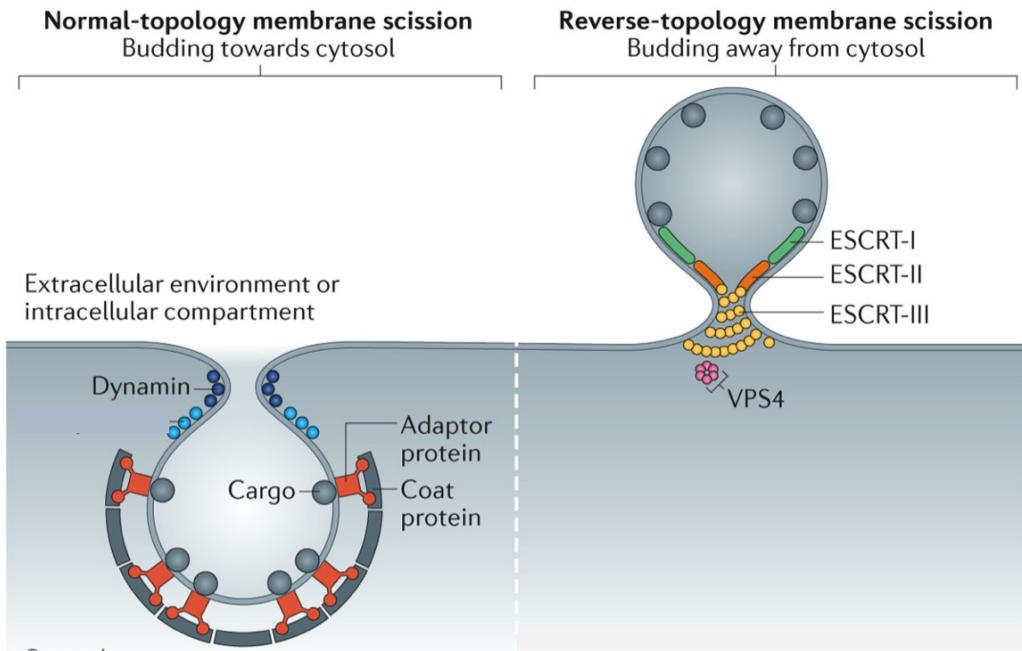
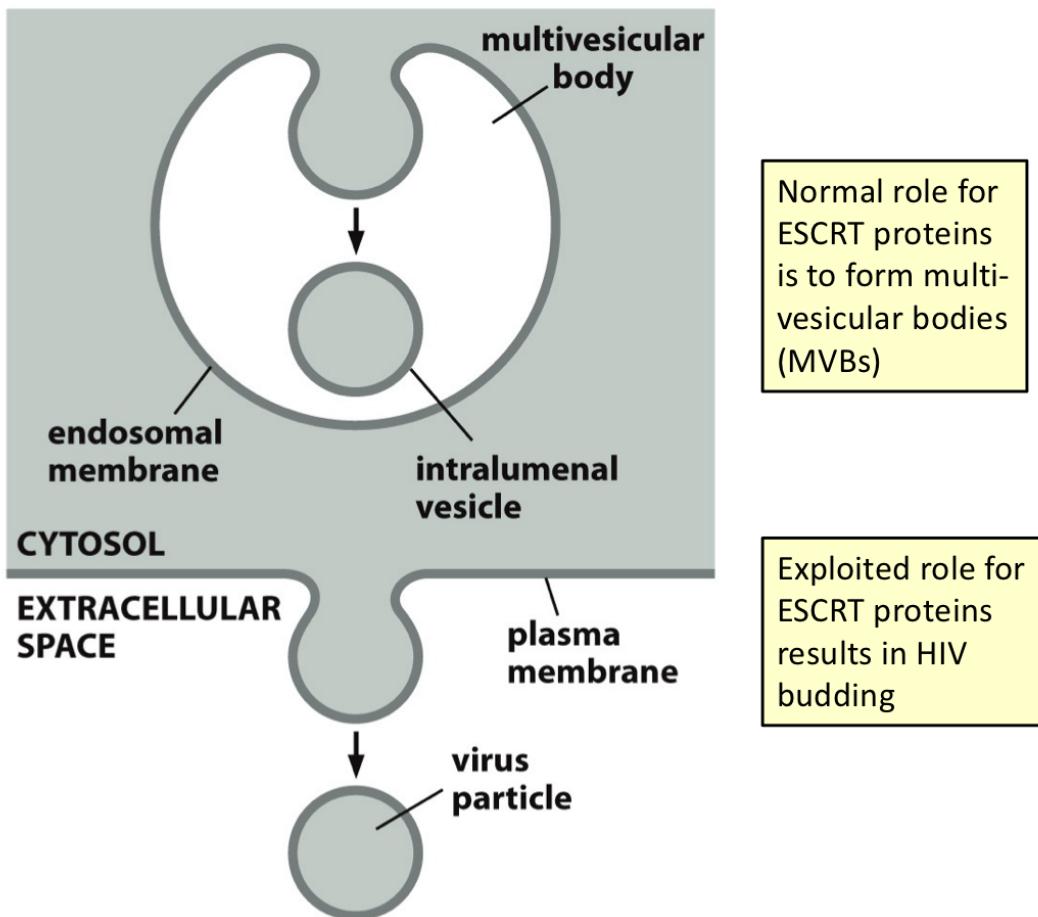
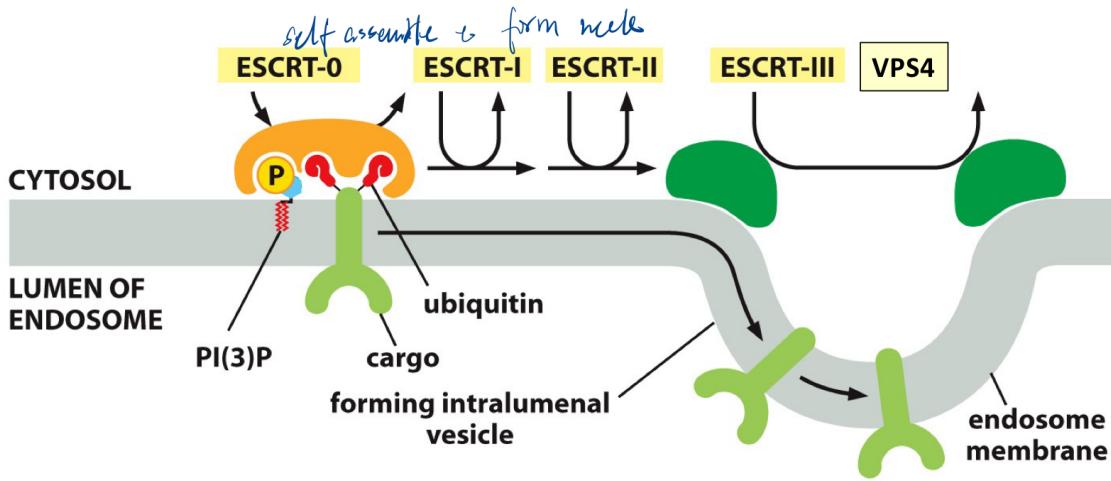


Figure 1

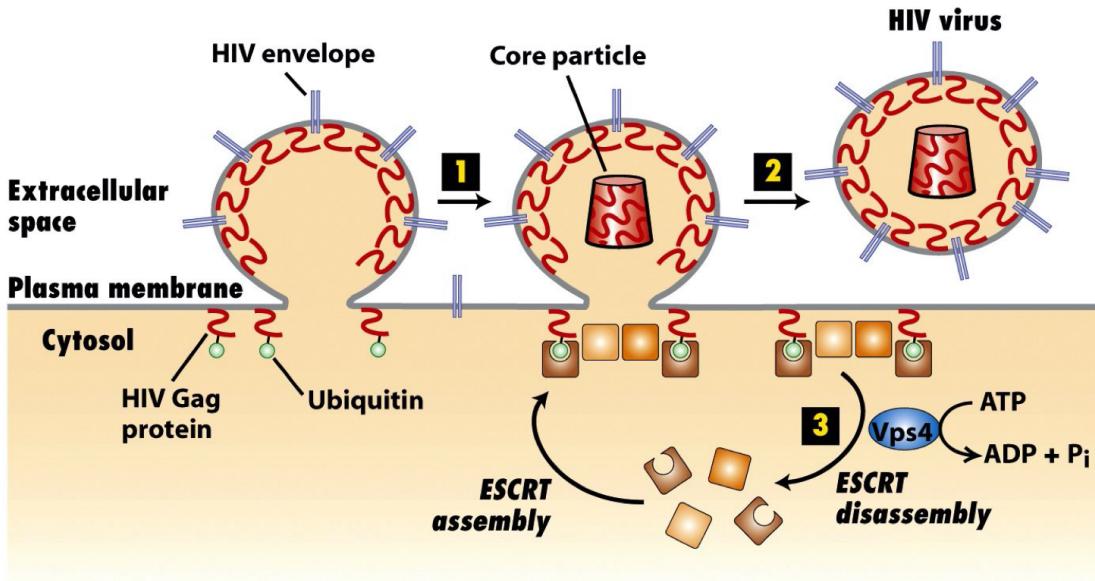
ESCRT-mediated membrane traffic







Normal role for ESCRTs is to target proteins with a ubiquitin (short amino acid sequence) tag into MVBs for degradation



HIV protein acquires a ubiquitin tag to use ESCRT assembly to bud into extracellular space

As discussed earlier, sorting into intraluminal vesicles requires one or multiple ubiquitin tags, which are added to the cytosolic domains of membrane proteins. These tags initially help guide the proteins into clathrin-coated

vesicles in the plasma membrane. Once delivered to the endosomal membrane, the ubiquitin tags are recognized again, this time by a series of cytosolic ESCRT protein complexes (ESCRT-0, -I, -II, and -III), which bind sequentially and ultimately mediate the sorting process into the intraluminal vesicles. Membrane invagination into multivesicular bodies also depends on a lipid kinase that phosphorylates phosphatidylinositol to produce PI(3)P, which serves as an additional docking site for the ESCRT complexes. For docking and vesicle invagination, ESCRT complexes require both PI(3)P and the presence of ubiquitylated cargo proteins to bind to the endosomal membrane. ESCRT-III forms large multimeric assemblies on the membrane that bend the membrane (Figure 13–60).

Mutant cells compromised in ESCRT function display signaling defects. In such cells, activated receptors cannot be down-regulated by endocytosis and packaging into multivesicular bodies. **The still-active receptors therefore mediate prolonged signaling, which can lead to uncontrolled cell proliferation and cancer.**

The ESCRT machinery that drives the internal budding from the endosome membrane to form intraluminal vesicles is also used in animal-cell cytokinesis and virus budding, which are topologically equivalent. In all three processes, budding occurs in a direction away from the cytosolic surface of the membrane (Figure 13–61A). ESCRT complexes are thought to have originated from similar components that mediate cell-membrane deformation during cytokinesis in archaea.

Although some viruses such as HIV hijack the host ESCRT machinery to bud directly out of the cell, other viruses escape using different mechanisms. For example, SARS-CoV-2, the virus that causes COVID-19, buds into the vesicular tubular clusters between the ER and Golgi apparatus, then uses the secretory pathway to exit the cell (Figure 13–61B). This budding reaction deforms membranes away from the cytosol but does not seem to use ESCRT machinery. Instead, viral proteins are thought to facilitate budding by a mechanism that is not well understood.