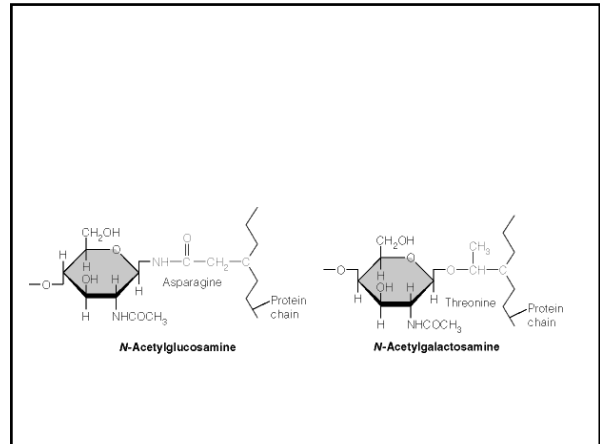
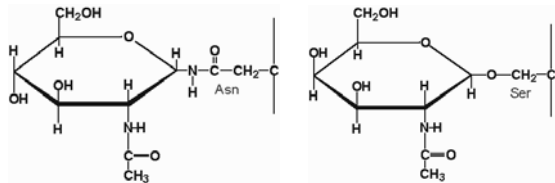


Protein modification and trafficking



There are two types of glycosylation



N-glycosidic bonds form via an N-glycosidic linkage is through the amide group of asparagine and the carboxyl group of N-acetylglucosamine

Protein Glycosylation

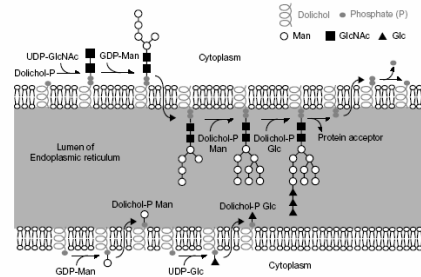
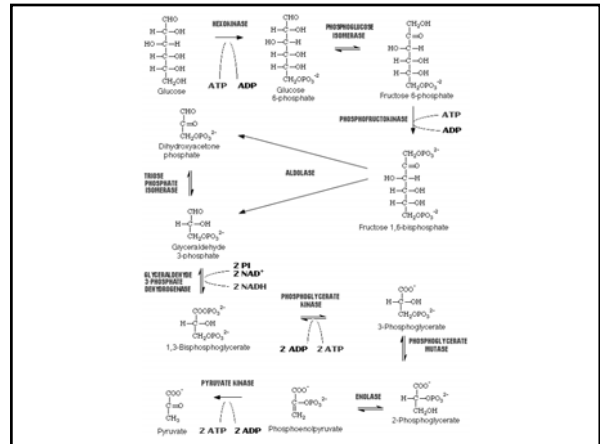
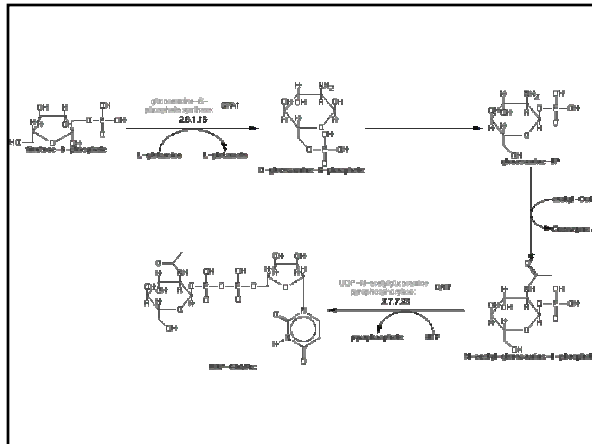
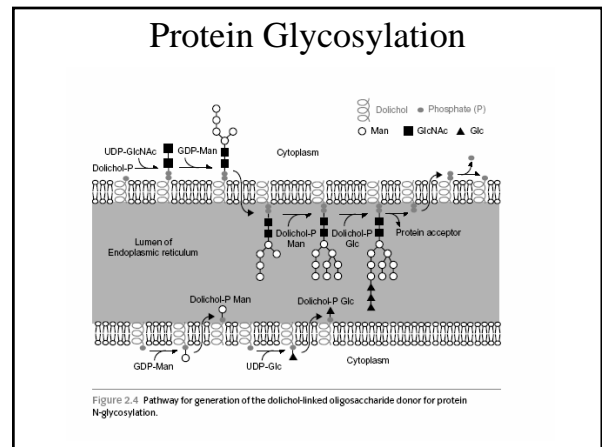
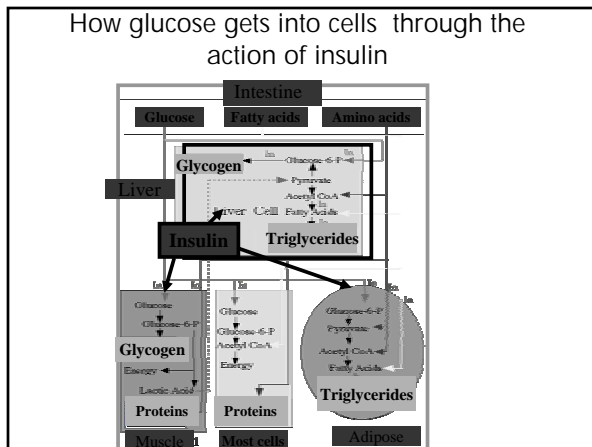
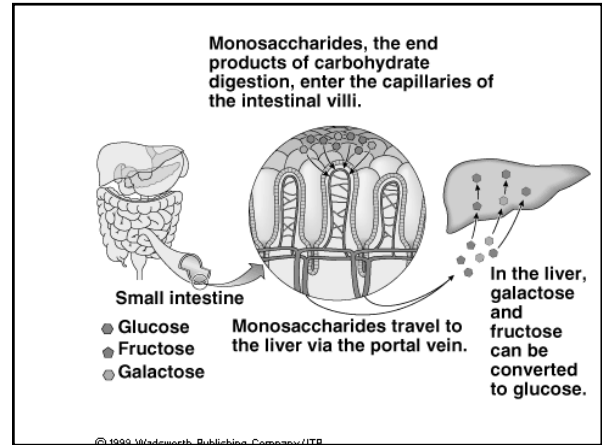
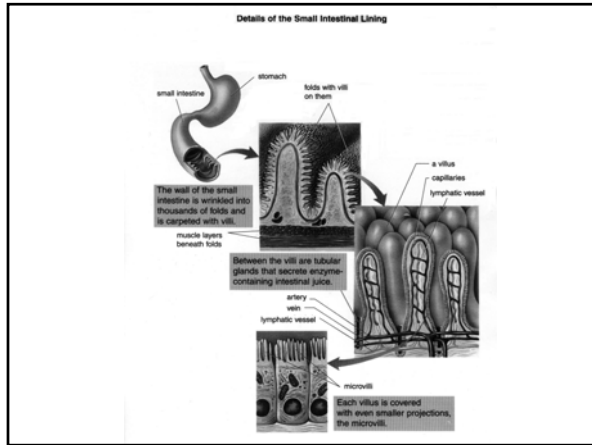
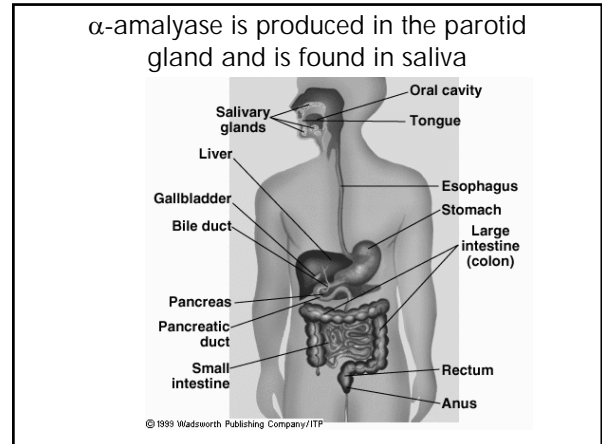
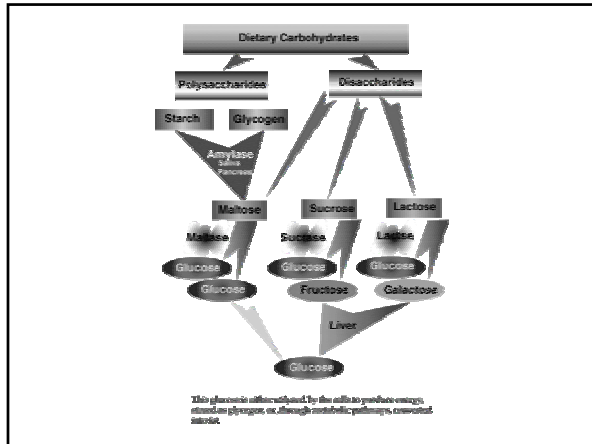


Figure 2.4 Pathway for generation of the dolichol-linked oligosaccharide donor for protein N-glycosylation.

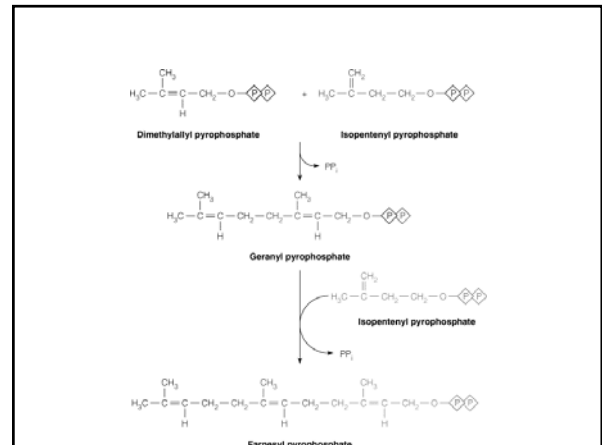
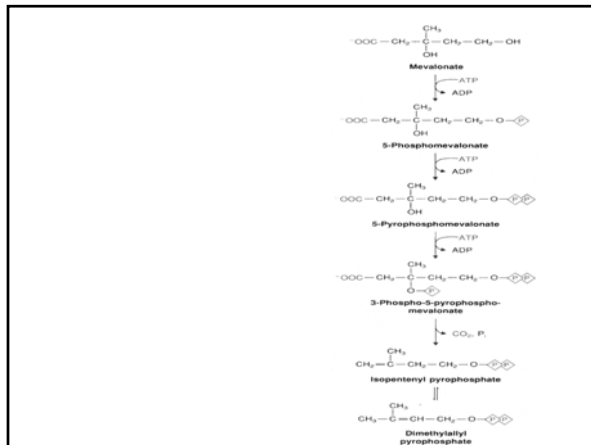
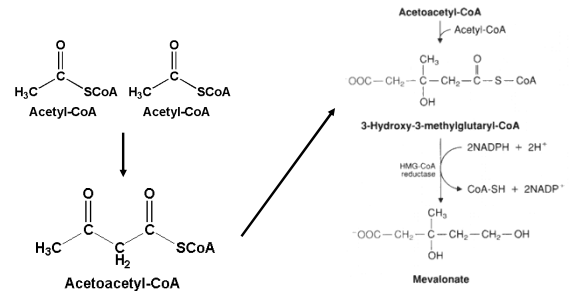




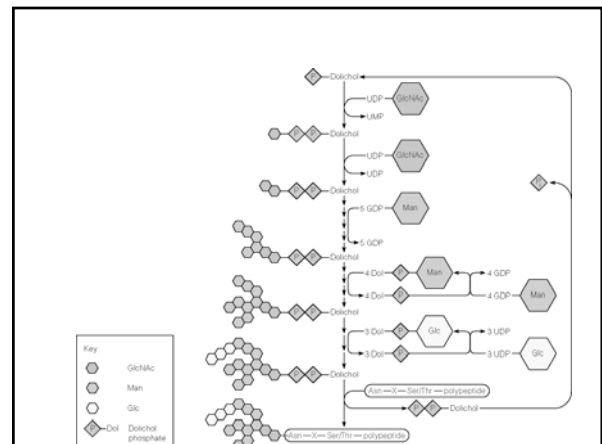
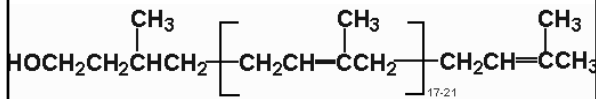
Where does Dolichol come from?

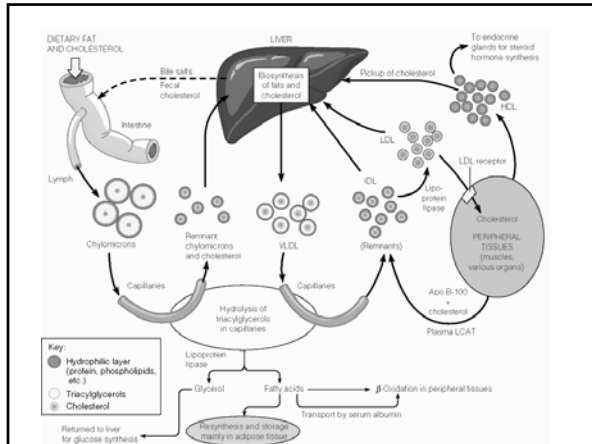
- Dolichol** is an isoprenoid compound synthesized by the same metabolic route as cholesterol. In vertebrate tissues, **dolichol** contains 18-20 isoprenoid units (90-100 carbons total). **Dolichol** is phosphorylated by a kinase that uses CTP to form **dolichol Phosphate**. **Dolichol phosphate** is the structure upon which the carbohydrate moieties of N-linked glycoproteins are built. After assembly on **dolichol phosphate**, the carbohydrate structure is transferred to an **asparagine** residue of a target protein having the sequence Asn-x-Ser/Thr, where X is any amino acid.

Cholesterol Biosynthesis



Dolichol





Disulfide bonds form between cysteines

- PDI protein disulfide isomerase works in the ER. In the cytosol most Cysteines are in the reduced state partly because of active oxygen radical scavengers. In the ER PDI works by forming disulfide bonds with the target protein and then transferring that bond to another cysteine within the target protein.

Further protein modification

Why glycosylation?

- Aids in proper protein folding
- Provides protection against proteases (e.g. lysosomal membrane proteins)
- Employed for signaling.
- Most soluble and membrane-bound proteins made in the ER are glycoproteins, in contrast to cytosolic proteins.
- Glycoprotein synthesis is a 3-part process:
 1. Assembly of the precursor oligosaccharide
 2. En-bloc transfer to the protein
 3. Modification of the oligosaccharide by removal of sugars

Where does glucose come from?

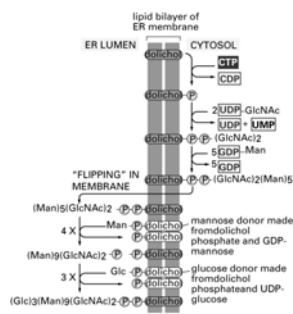
- Starch is the major source of dietary glucose. The enzymes responsible for starch degradation are called *amylases*. Other sources of glucose are sucrose, a disaccharide glucose-fructose from fruits, and lactose, a glucose-galactose disaccharide from milk. Only monosaccharide species like glucose, fructose and galactose can be absorbed via active membrane transport systems. Special intestinal glucosidases split the disaccharides into their monosaccharide components. Maltose is hydrolyzed by *isomaltase* (oligo-1,6-glucosidase, E.C. 3.2.1.10) and, with lower efficacy, by *sucrase* (sucrose alpha-glucosidase, E.C. 3.2.1.48). Lactose intolerance comes from a lack of *lactase* in many adults, causing an accumulation of milk sugar with consequences such as dehydration.

Protein glycosylation

I. Assembly of the precursor oligosaccharide...

IMPORTANT POINTS:

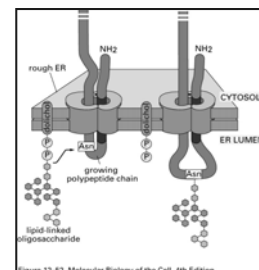
- Assembly takes place on the carrier lipid dolichol, anchored in the ER membrane.
- A pyrophosphate bridge joins the 1st sugar to the dolichol.
- Sugars are added singly and sequentially.
- After the two N-acetylglucosamines are added, the assembly flips from the cytosolic side to the ER lumen.
- Nine mannose and three glucose molecules are added, totaling 14 sugars.



Now in the second step, attachment

2. En-bloc transfer of the oligosaccharide to the protein

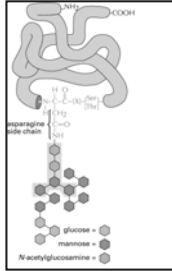
- One step transfer, catalyzed by oligosaccharyl transferase, which is bound to the membrane at the translocator.
- Covalently attached to certain asparagines in the polypeptide chain (said to be "N-linked" glycosylation).
- Attaches to NH₂ side chain of Asn but only in the context: **Asn-x-Ser or Asn-x-Thr**



Finally modification of oligosaccharide

Modification of the oligosaccharide by removal of sugars...

- Three glucoses and one mannose are removed sequentially in the ER.



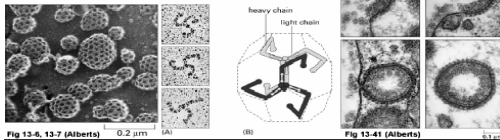
Transport from the ER to Golgi

- Appropriately modified proteins leave the ER and travel to the Golgi Apparatus.
- They travel in membrane vesicles that arise from special regions of membranes that are coated by proteins.
- There are three types of coated vesicles that are well characterized, clathrin-coated, COPI-coated and COPII-coated vesicles.
- COPI and COPII act mainly in ER or Golgi cisternae.
- Clathrin acts in Golgi or plasma membranes.

Clathrin coated vesicles

Clathrin vesicles mediate multiple types of intracellular transport

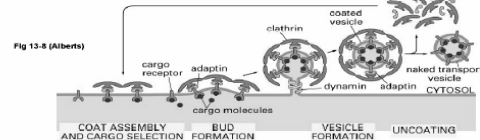
- receptor mediated endocytosis (e.g., LDLR, TTR)
- TGN trafficking (e.g., Golgi/hotspot) (e.g., MGPR)



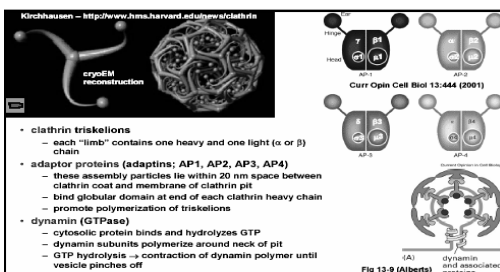
Clathrin cycle

Clathrin cycle

- recruitment of adaptins
- clathrin assembly
- dynamin recruitment
- coated pit invagination
- coated pit constriction w/ dynamin redistribution from lattice to neck
- coated vesicle budding
- clathrin depolymerization
- release of adaptins



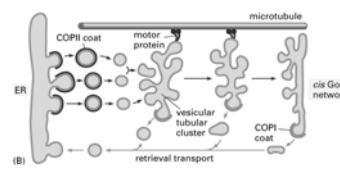
Clathrin adaptins and dynamin



- clathrin triskelions
 - each "limb" contains one heavy and one light (α or β) chain
- adaptor proteins (adaptins; AP1, AP2, AP3, AP4)
 - these assembly particles lie within 20 nm space between clathrin coat and membrane of clathrin pit
 - bind globular domain at end of each clathrin heavy chain
 - promote polymerization of triskelions
- dynamin (GTPase)
 - cytosolic protein binds and hydrolyzes GTP
 - dynamin subunits polymerize around neck of pit
 - GTP hydrolysis \rightarrow contraction of dynamin polymer until vesicle pinches off

Transport from the ER to the Golgi

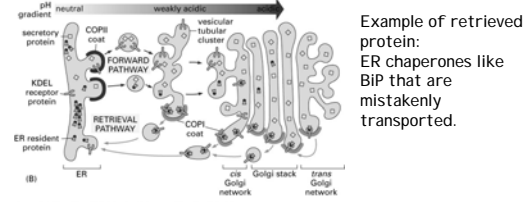
When the protein is properly folded, COPII coated vesicles transport the proteins via the vesicular tubular cluster (vtc) to the cis-Golgi network.



- The COPII coating is removed (Sar1 hydrolyzes GTP) and the vesicles fuse with each other to form the vtc.
- The vtc is motored along microtubules that function like railroad tracks.
- The vtc fuses with the cis-Golgi network.

Figure 13-20 part 2 of 2, Molecular Biology of the Cell, 4th Edition.

Some proteins exiting the ER are returned to the ER by COPI coated vesicles. These proteins are identified by the presence of specific signal sequences that interact with the COPI vesicles or associate with specific receptors.



Example of retrieved protein: ER chaperones like BIP that are mistakenly transported.

Figure 13-21 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

This example describes the situation of BIP. BIP has the signal sequence, KDEL. When BIP escapes the ER, it associates with the KDEL receptor. The slightly acid environment of the vtc and Golgi favor this association. When the returning vesicle fuses with the ER, the neutral pH of the ER causes BIP to dissociate from the receptor.

Proteins exiting the ER join the Golgi apparatus at the cis Golgi network. The Golgi apparatus consists of a collection of stacked compartments.

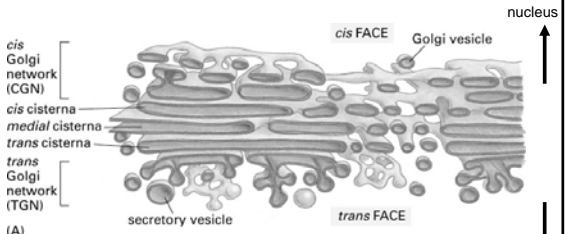


Figure 13-22 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Cell membrane

The Golgi Apparatus has two major functions:

1. Modifies the N-linked oligosaccharides and adds O-linked oligosaccharides.
2. Sorts proteins so that when they exit the trans Golgi network, they are delivered to the correct destination.

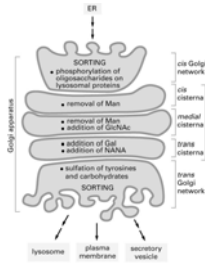


Figure 13-28. Molecular Biology of the Cell, 4th Edition.

Modification of the N-linked oligosaccharides is done by enzymes in the lumen of various Golgi compartments.

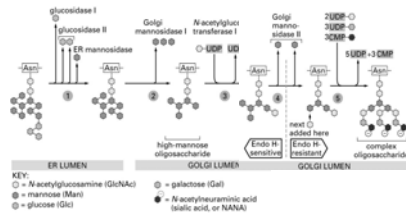


Figure 13-29 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

While N-linked glycosylation appears to function in protein-folding in the lumen of the ER, the function of the oligosaccharide modifications occurring in the Golgi is largely unknown.

One ultimate destination of some proteins that arrive in the TGN is the lysosome. These proteins include acid hydrolases.

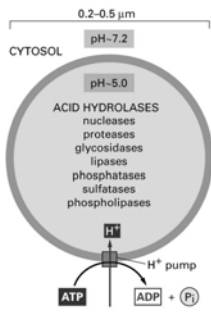


Figure 13-31. Molecular Biology of the Cell, 4th Edition.

Lysosomes are like the stomach of the cell. They are organelles surrounded by a single membrane and filled with enzymes called acid hydrolases that digest (degrade) a variety of macromolecules. A vacuolar H⁺ ATPase pumps protons into the lysosome causing the pH to be ~5.

The macromolecules that are degraded in the lysosome arrive by endocytosis, phagocytosis, or autophagy.

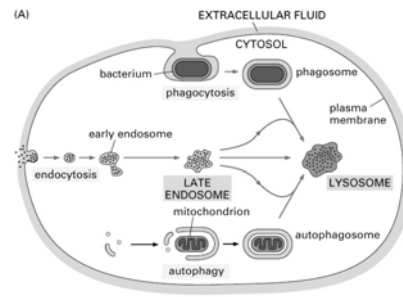


Figure 13-35 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

The acid hydrolases in the lysosome are sorted in the TGN based on the chemical marker mannose 6-phosphate.

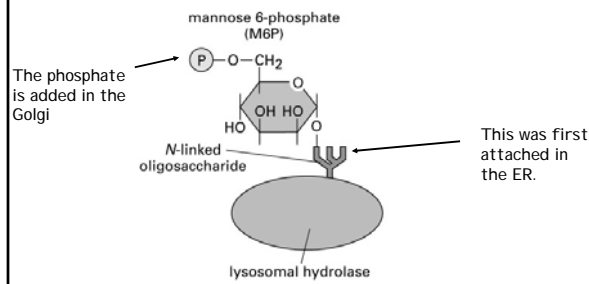


Figure 13-36. Molecular Biology of the Cell, 4th Edition.

This was first attached in the ER.

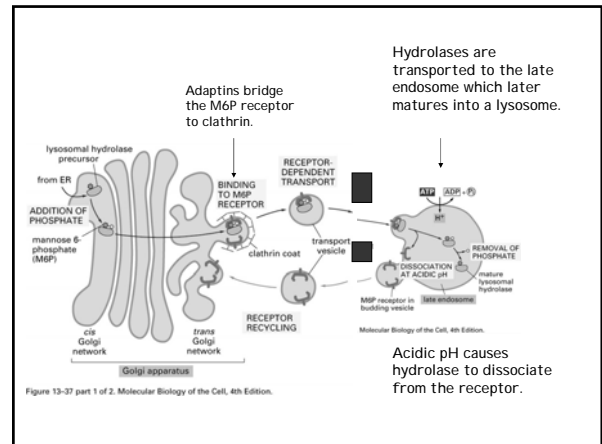


Figure 13-37 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Hydrolases are transported to the late endosome which later matures into a lysosome.

Acidic pH causes hydrolase to dissociate from the receptor.

The creation of the M6P marker in the Golgi relies on recognition of a signal patch in the tertiary structure of the hydrolase.

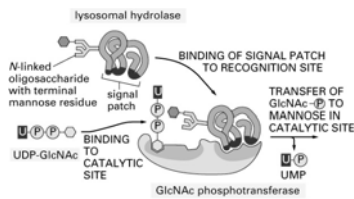
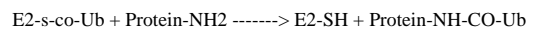
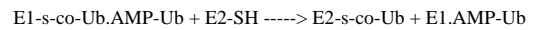
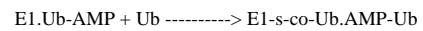
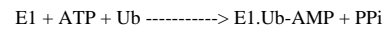


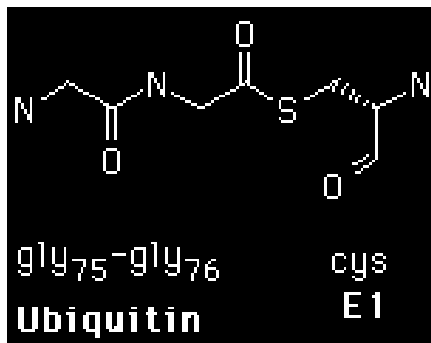
Figure 13-38 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Patients with a disease called inclusion-cell disease have cells lacking hydrolases in their lysosomes. Instead, the hydrolases are found in the blood. These patients lack GlcNAc phosphotransferase. Without the M6P-tag, the acid hydrolases are transported to the plasma membrane instead of the late endosome.

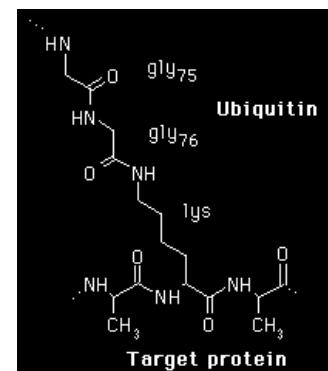
Ubiquitin pathway for protein degradation

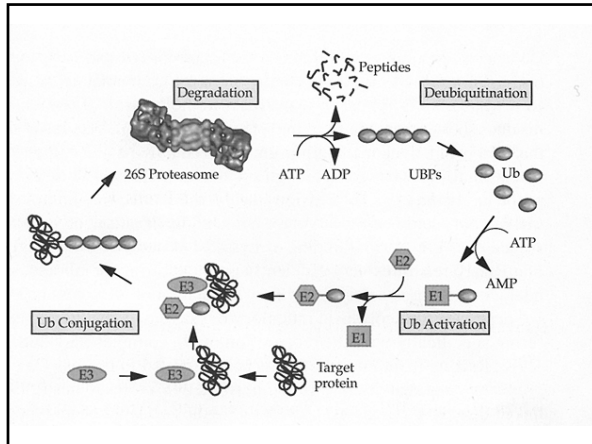


Thioester bond



Isopeptide bond





Protein degradation

- For some proteins, more than 80% of peptides may not fold properly. These are removed from the ER and degraded.
- Retrotranslocation (or dislocation)
- Uses the same Sec61 translocator
- N-glycanase removes the oligosaccharide.
- Ubiquitin chain added to protein which marks it for degradation in the proteasome.

Figure 13-46, Molecular Biology of the Cell, 4th Edition.

END