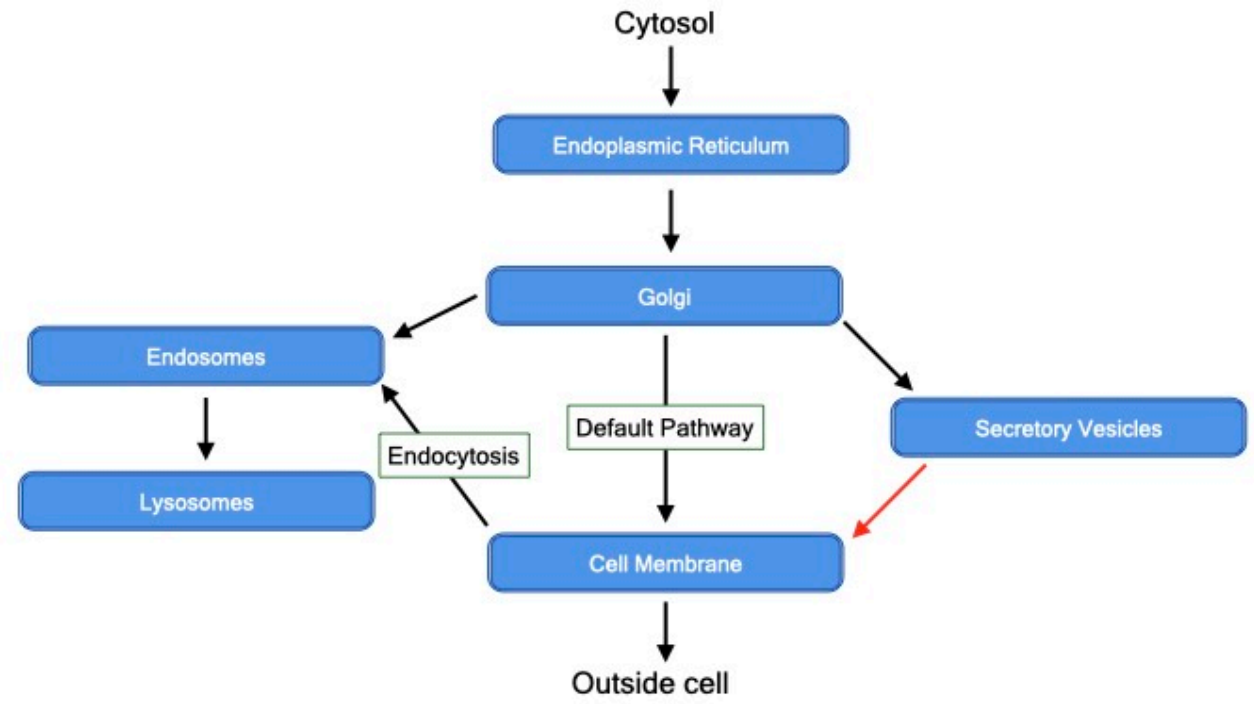
Secretory Pathway

Scientific Foundations

The importance of the cell membrane to the viability and function of cells raises a couple of important questions. First, how do the protein channels, receptors, and adhesion proteins get into the membrane when proteins cannot diffuse across a membrane? Second, how do cells replace those phospholipids and proteins in a cell membrane that are damaged and have loss part or all of their function?

The secretory pathway allow cells to deliver phospholipids and proteins to the cell membrane and some organelles (e.g., lysosome, secretory granules). The secretory pathway comprises a set of distinct membrane-bound organelles that synthesize proteins and phospholipids, modify them, and delivery them to their final destination.

The diagram below provides an overview of the membrane-bound organelles in the secretory pathway. The entry point for the secretory pathway is the endoplasmic reticulum. Here, proteins are synthesized and inserted into or across the phospholipid bilayer. In addition, most steps in the synthesis of phospholipids occurs in the endoplasmic reticulum. From the endoplasmic reticulum, proteins and phospholipids are delivered to the Golgi.



Secretory pathway delivers proteins and lipids to the cell membrane and organelles.

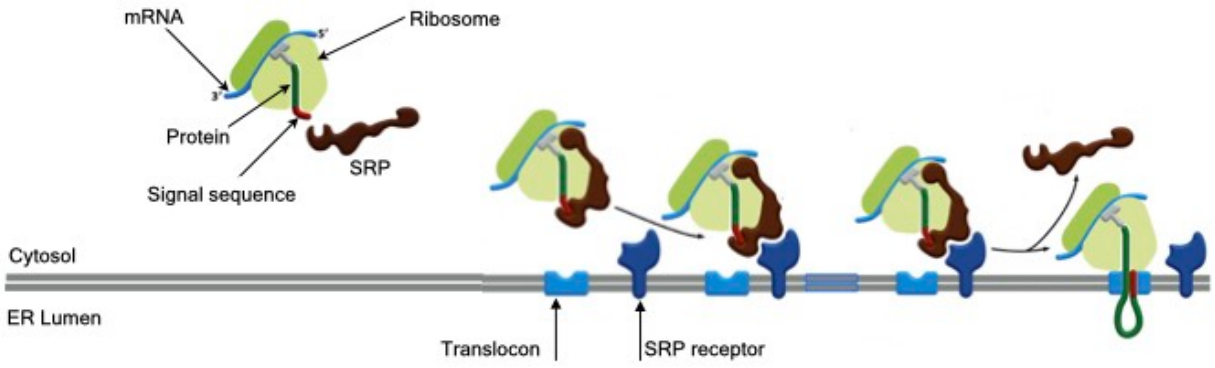
The Golgi performs two essential functions. First, it modifies sugar side chains on proteins that were added in the endoplasmic reticulum. Second, it sorts proteins to their final destination, often by recognizing signal sequences, which are short stretches of amino acids within proteins. By default, proteins and lipids are delivered to the cell membrane but can be diverted to internal organelles, such as lysosomes or secretory vesicles. Secretory vesicles store proteins and other molecules within a membrane organelle in the cytoplasm of cells. When stimulated, the vesicles fuse with the cell membrane to release their contents to the external environment.

In addition to delivering material to the cell membrane, cells can internalize portions of their cell membranes through a process called endocytosis. Endocytosis allows cells to remove specific proteins from the cell membranes or take up soluble material from the external environment.

Protein Import into the Endoplasmic Reticulum

The first step in the secretory pathway is the entry of proteins into the endoplasmic reticulum (ER). Protein import into the ER requires coordination between the translation of protein from mRNA and translocation of that protein across the membrane the ER.

All proteins are synthesized from mRNA by ribosomes in the cytosol (fluid portion of cytoplasm) of cells. Proteins destined for the secretory pathway contain a signal sequence at their N-terminus. When this signal sequence emerges from the ribosome, a complex of 6 proteins and one RNA, called the signal-recognition particle (SRP), binds to the signal sequence. After binding to the signal sequence, SRP pauses translation of the protein.



Secretory proteins are translated and translocated across the ER membrane.

The ribosome with SRP is next bound to the surface of the ER membrane through the interaction between SRP and a receptor embedded in the ER membrane called SRP receptor. The protein being synthesize by the ribosome cannot diffuse across the phospolipid bilayer of the ER membrane, so SRP receptor resides next to a protein channel called the translocon. The translocon

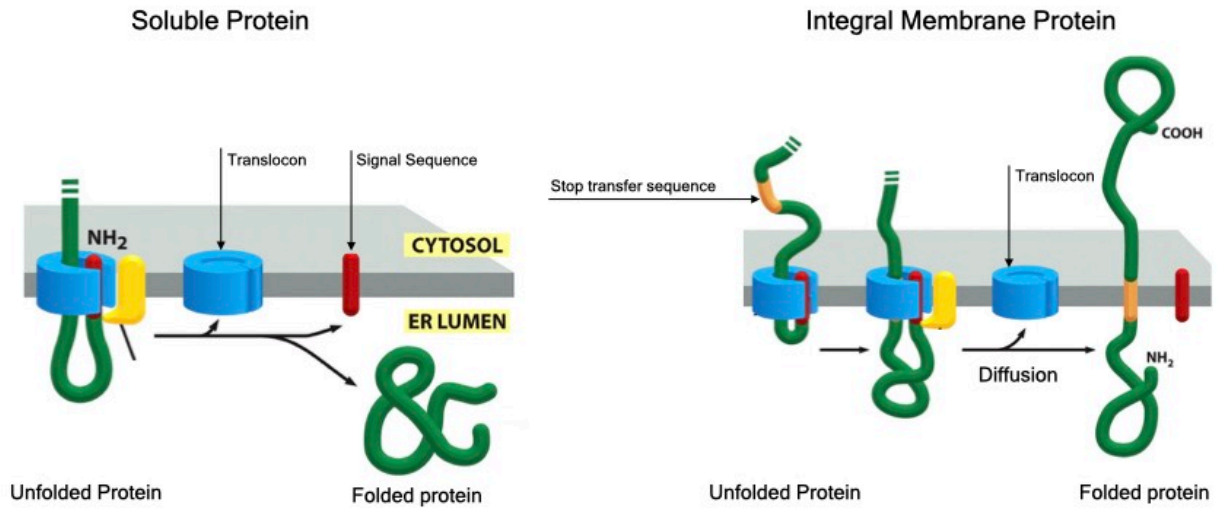
forms an aqueous pore across the ER membrane through which a protein can pass from the cytosol into the ER.

The interaction between SRP and its receptor causes SRP to release from the nascent protein and ribosome. This allows translation to continue and the energy of translation pushes the protein through the translocon into the ER.

Soluble and Integral Membrane Proteins

Synthesis and translocation of proteins across the ER membrane can generate both soluble proteins that reside entirely within the lumen of the ER and integral membrane proteins that span the ER membrane at least once and up to several times. Usually, soluble proteins are secreted into the external environment, whereas integral membrane proteins that reach the cell membrane make up the receptors, channels, pumps and adhesion proteins that generate specific functions for the cell.

Soluble proteins contain one signal sequence that initiates translocation of the protein entirely across the ER membrane. After translocation, the bulk of the protein is cleaved from the signal sequence which releases the protein into the lumen of the ER.



Translocation can generate soluble and integrated membrane proteins.

Integral membrane proteins also contain a signal sequence that mediates translocation but contain an additional signal sequence called a stop-transfer sequence. The stop transfer sequence pauses translocation through the translocon and allows the protein to diffuses out of the translocon with the stop transfer sequence spanning the lipid bilayer. The signal sequence is then cleaved from the protein.

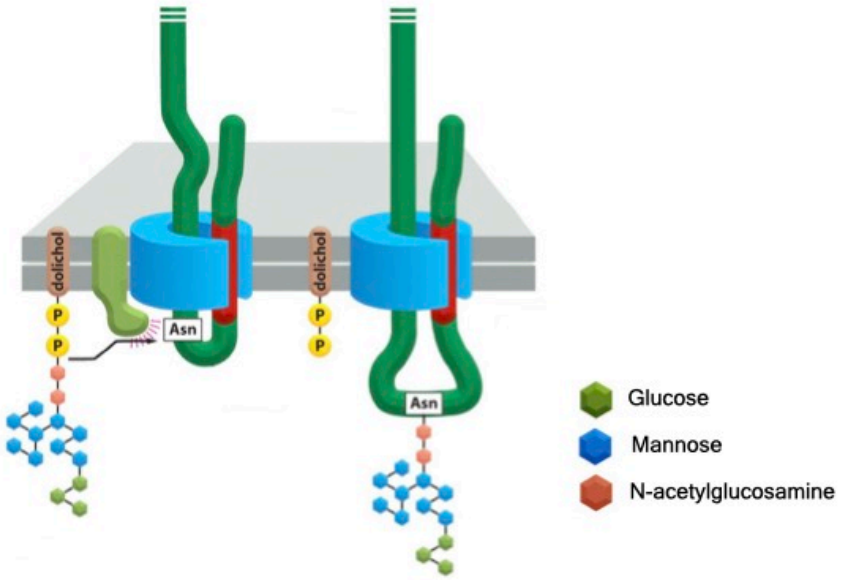
Proteins that span the membrane more than once contain a start transfer sequence downstream of the stop-transfer sequence that reinitiates translocation. A second stop-transfer sequence stops translocation and forms the second membrane-spanning region. These alternating start and stop transfer sequences can be repeated several times to generate proteins that span the ER membrane several times.

Protein Folding

The pore in the translocon can only accommodate proteins in an unfolded state. Proteins moving through the translocon must fold into a proper structure before they are allowed to leave the ER (the ER has mechanisms to prevent unfolded protein from exiting). Mutations in proteins that inhibit their folding or high levels of synthesis of secreted protein can accumulate unfolded protein in the ER, which can lead to cell death. You will encounter several diseases where one of the causes is due to accumulation of unfolded protein.

Protein Glycosylation

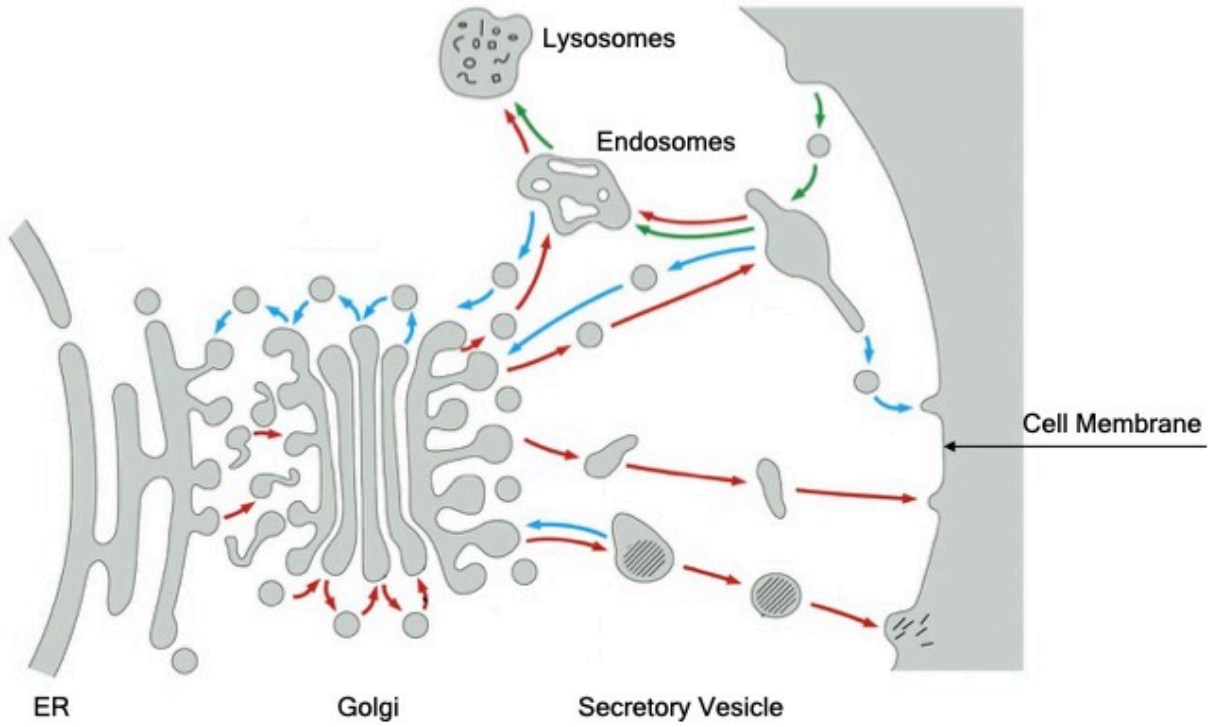
Many proteins that enter the ER are modified by the transfer of a block of sugar residues onto an asparagine residue in the protein. The block of sugars is covalently attached to a nitrogen atom in asparagine and is therefore called N-linked glycosylation. The block of sugars contains a mix of glucose, mannose and N-acetylglucosamine that are assembled into a tree-like structure. The block of sugars serve several functions including protein localization (see below), protein folding and cell adhesion.



Many proteins are modified through glycosylation in the ER.

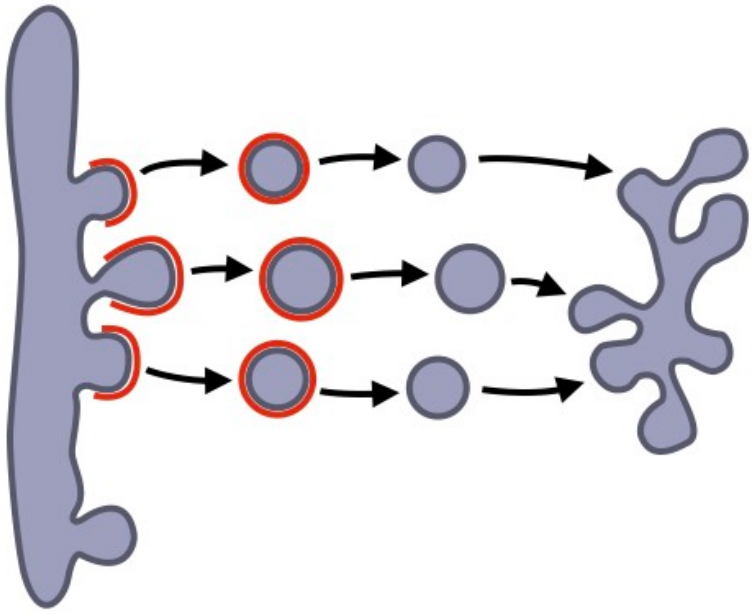
Vesicular Transport

After proteins are imported into the ER, they must be delivered to their appropriate location (e.g. cell membrane, lysosome, etc.) via the secretory pathway. Trafficking of proteins between organelles is mediated by small, membrane-bound vesicles that form from one organelle and then fuse with another organelle.



Vesicles mediate transport between membrane-bound compartments.

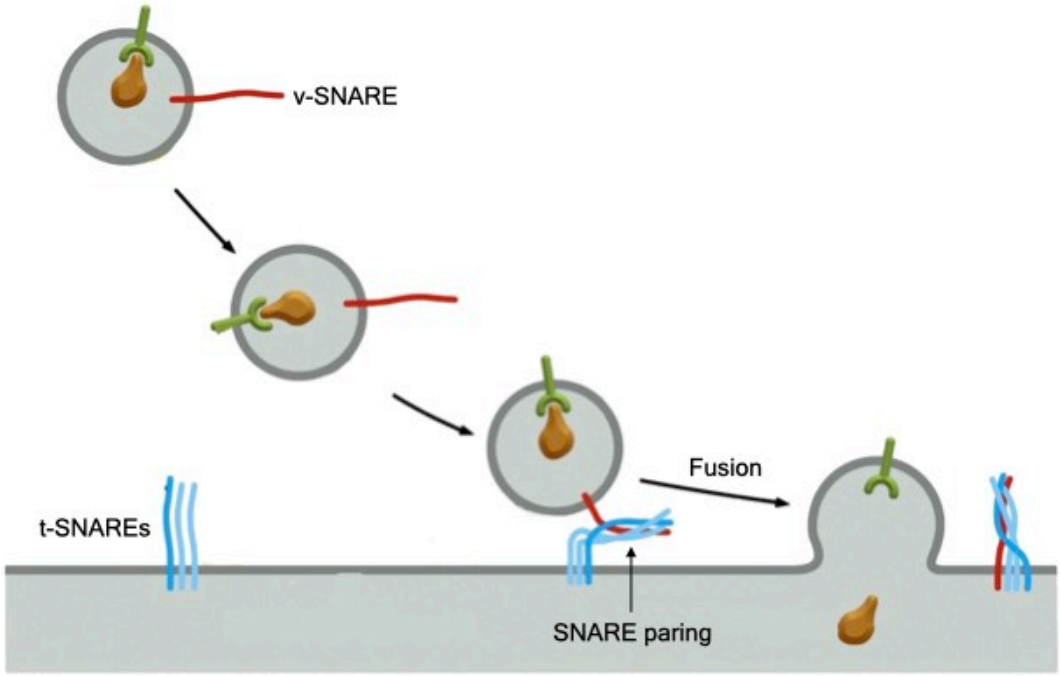
Vesicular transport between organelles consists of three steps. First, vesicles bud from one organelle (e.g ER). The vesicle is then targeted to the appropriate organelle (e.g Golgi). Finally, the vesicle fuses with the target organelle to mix its contents with the contents of the target organelle. The formation of a vesicle also includes a mechanism for cargo selection because only a subset of proteins in an organelle should leave that organelle to move to another organelle. Some proteins are considered resident proteins because they perform essential functions in an organelle. The formation of vesicles and cargo selection are mediated by proteins that form a coat around the vesicle. After formation of the vesicle, the coat falls off allowing the vesicle to fuse with its target organelle.



Vesicular transport consists of budding, targeting and fusion.

Vesicle Targeting and Fusion

Once vesicles form from one organelle, they need to fuse with another organelle or the cell membrane to deliver their cargo. There are many different membrane-bound organelles in the cell, so vesicles need a mechanism to recognize the correct target organelle.



SNAREs target vesicles to their destination and mediate fusion of membranes.

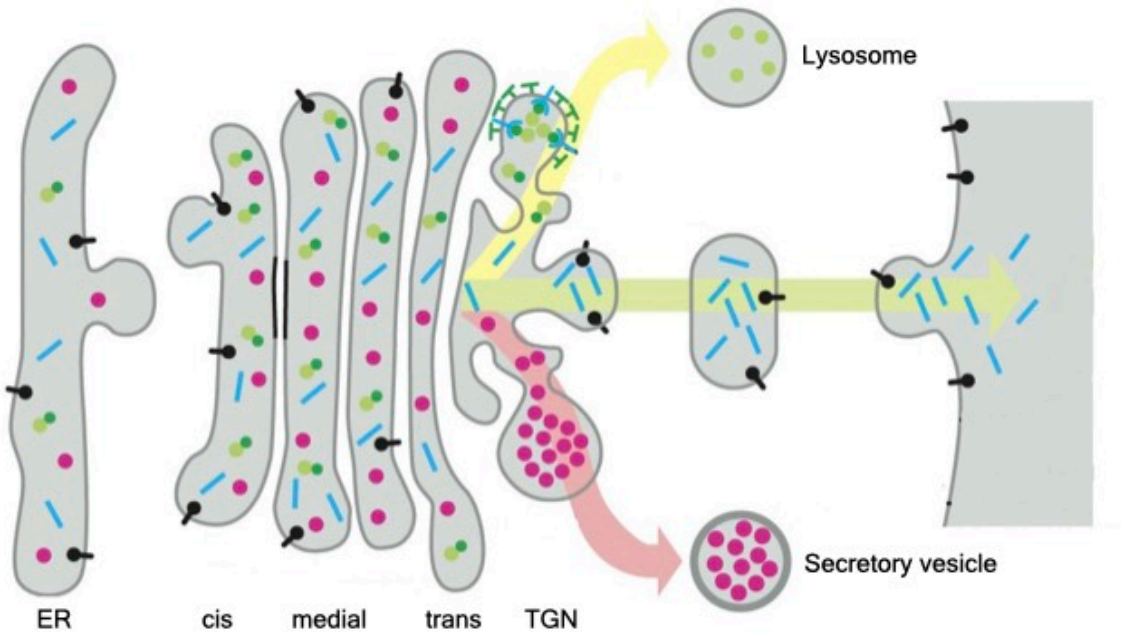
A large family of proteins called SNAREs mediates fusion between vesicles and organelle. SNAREs are transmembrane proteins that reside in both organelles and vesicles. SNAREs in vesicles are

called v-SNAREs and SNAREs in organelles are called t-SNAREs (for target). There are over 35 different SNARE proteins allowing the cells to mark different types of organelles and vesicles with a unique set of SNAREs. Certain v-SNAREs and t-SNAREs interact or pair to generate a specific interaction between a vesicle with its target organelle. The energy of pairing between SNAREs is thought to drive the fusion of the vesicle membrane and organelle membrane. Fusion mixes the contents of the vesicle with the target organelle.

Protein Sorting in the Secretory Pathway

By default, proteins that enter the secretory pathway are delivered to the cell membrane. If the protein is an integral membrane protein, it will reside in the cell membrane (e.g., receptor, channel). If the protein is soluble (dœs not span the membrane), it will be released to the surrounding environment.

Proteins that need to be delivered to another location (e.g., intracellular organelle) are sorted in the Golgi. The Golgi comprises a series of disc-shaped, membrane-bound organelles called cisternæ. Proteins from the ER are delivered to the cis-side and proteins leave the Golgi from the trans-side. If a protein contains a certain signal sequence, the Golgi will divert the protein from delivery to the cell membrane and target the protein to its correct destination.

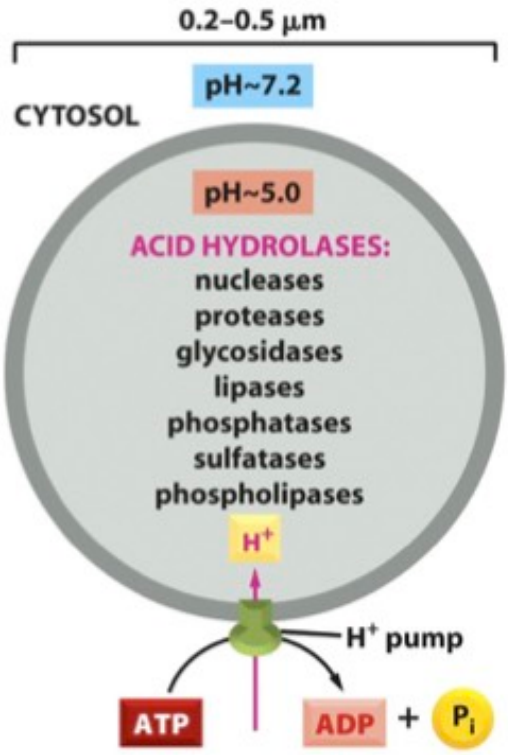


Proteins are sorted in trans-Golgi network to their final destinations.

Protein Trafficking to Lysosomes

Let's look at important example of how proteins are targeted to an intercellular organelle, the lysosome. Lysosomes are membrane-bound organelles that digest cellular and foreign material.

The lumen of the lysosome contains many protein enzymes that breakdown different macromolecules. These enzymes are active at low pH, and proton pumps in the membrane of lysosomes acidifies its lumen. The advantage of using enzymes that are active at low pH is it keeps those enzymes inactive until they reach the lysosome. To perform its myriad of activities the lysosome requires the secretory pathway to deliver a constant supply of several different digestive enzymes. If lysosomes lack one or more of these enzymes, then biological material accumulates in the lysosome, and the lysosome enlarges.

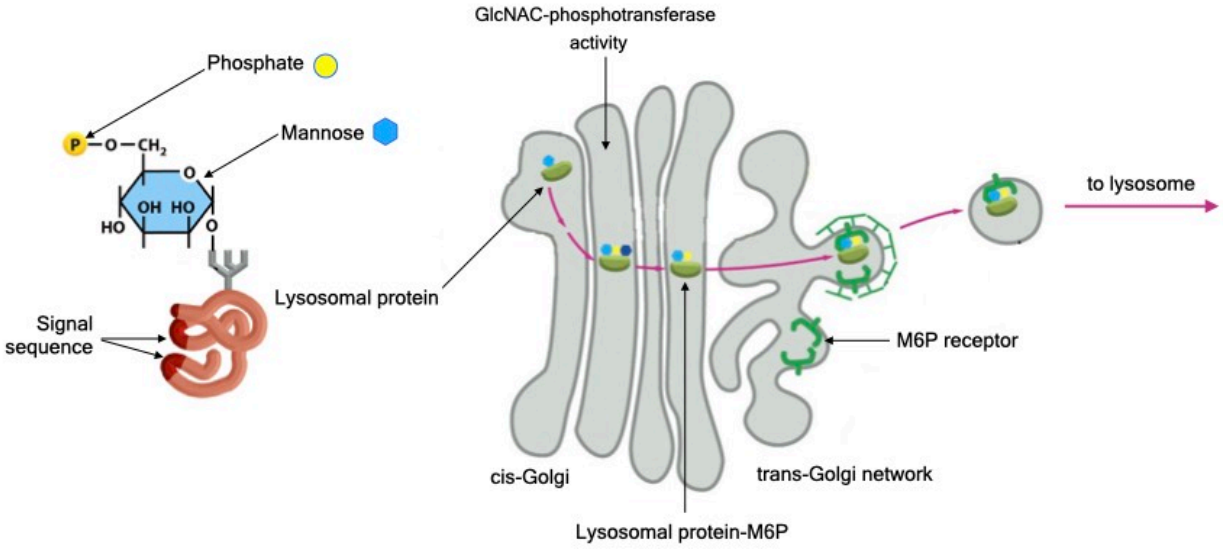


Lysosomes contain many digestive enzymes that are active at low pH.

Many enzymes are targeted to the lysosome via a unique sugar modification called mannose 6-phosphate. Recall that proteins are glycosylated in the ER, and mannose 6-phosphate will be added to proteins that are meant for the lysosome.

Proteins destined for the lysosome contain a signal sequence that is recognized by an enzyme in the Golgi called GlcNAC-phosphotransferase. The enzyme catalyzes the transfer of a sugar called N-acetylglucosamine with a phosphate onto the mannose residue of the sugar side chain of the lysosomal protein. The N-acetylglucoamine is eventually removed, leaving mannose-phosphate on the lysosomal protein.

A receptor in the TGN recognizes proteins with mannose-6-phosphate and recruits those proteins into vesicles that will be delivered to lysosomes.

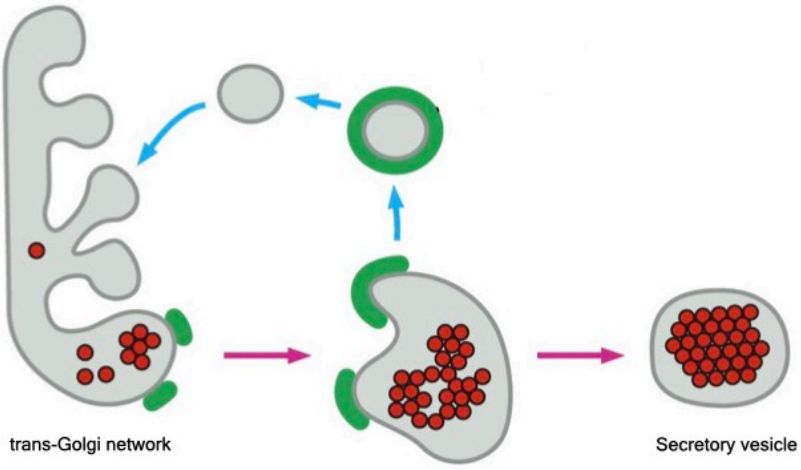


Mannose-6-phosphate targets proteins to lysosomes.

Secretory vesicles

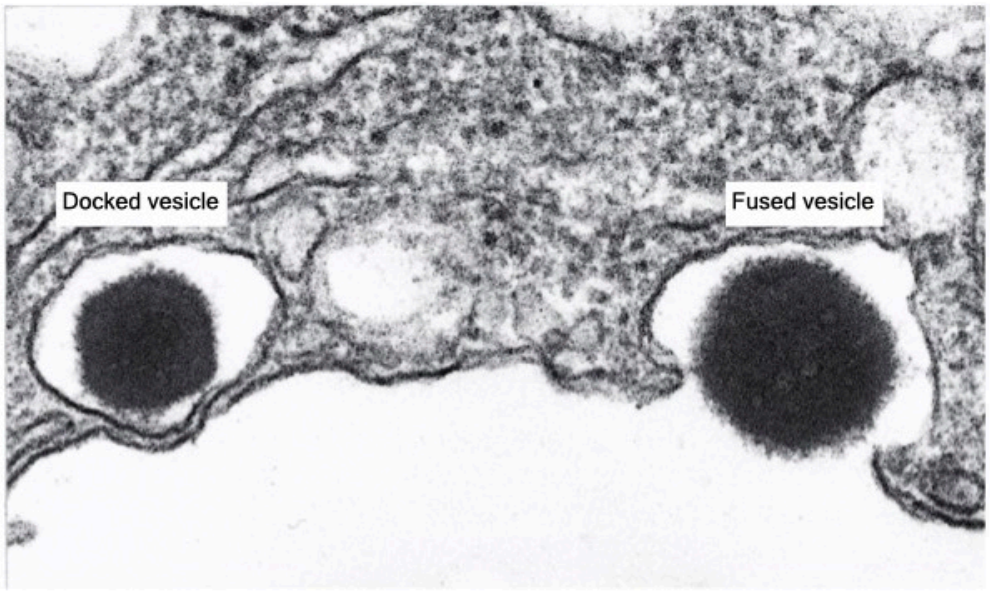
Some cells release proteins and peptides to the external environment and store those components in intracellular vesicles called secretory vesicles. This allows cells to accumulate a large amount of material and quickly release that material upon receiving a signal to do so.

The mechanism of targeting protein to secretory vesicles is less clear than how proteins are trafficked to lysosomes. The process appears to involve a signal patch on proteins that induces them to cluster in the TGN. The aggregated protein is selected by an unknown mechanism into vesicles that bud from the TGN. After budding from the TGN, secretory vesicle undergo a maturation process during which the content of the secretory vesicle is concentrated. Secretory granules do not immediately fuse with the cell membrane but remain in the cytoplasm until an appropriate signal to fuse is received by the cell.



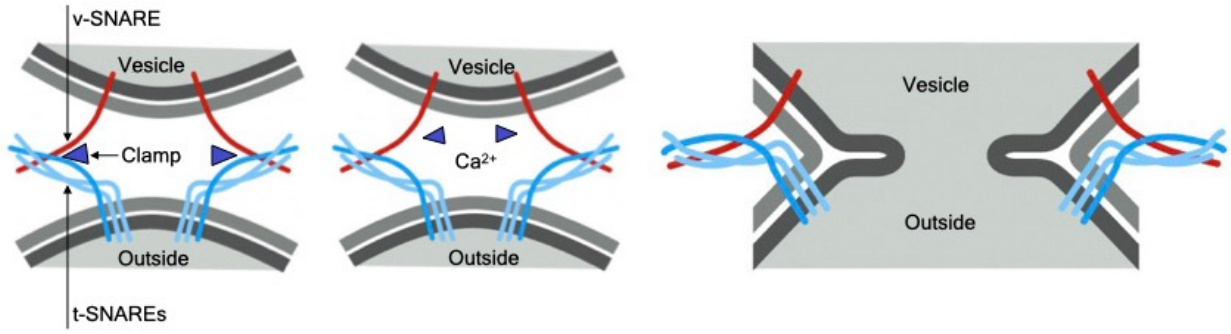
Secretory vesicles accumulate aggregated proteins in TGN and undergo maturation.

Some secretory vesicles dock at the cell membrane in an arrested state of fusion. Fusion with the cell membrane is completed only when the cell receives an appropriate signal. This is an example of regulated fusion that is common in a variety of different cells.



Some secretory vesicles dock at the cell membrane awaiting a signal to fuse.

The mechanism of one type of regulated fusion is mediated by the SNARE proteins. The v-SNARE in the granule and t-SNAREs in the cell membrane interact but are prevented from completely pairing by an inhibitory protein called a clamp. When a signal is received, there is an increase in cytosolic calcium that dissociates the clamp from the SNAREs. Now the SNAREs can complete their pairing to drive fusion of the vesicle with the cell membrane. Docking of the granule at the cell membrane allows for rapid release of the vesicle contents in response to stimuli and is the mechanism by which neurons release neurotransmitter.



Calcium-sensitive clamps inhibit SNARE pairing to arrest fusion.

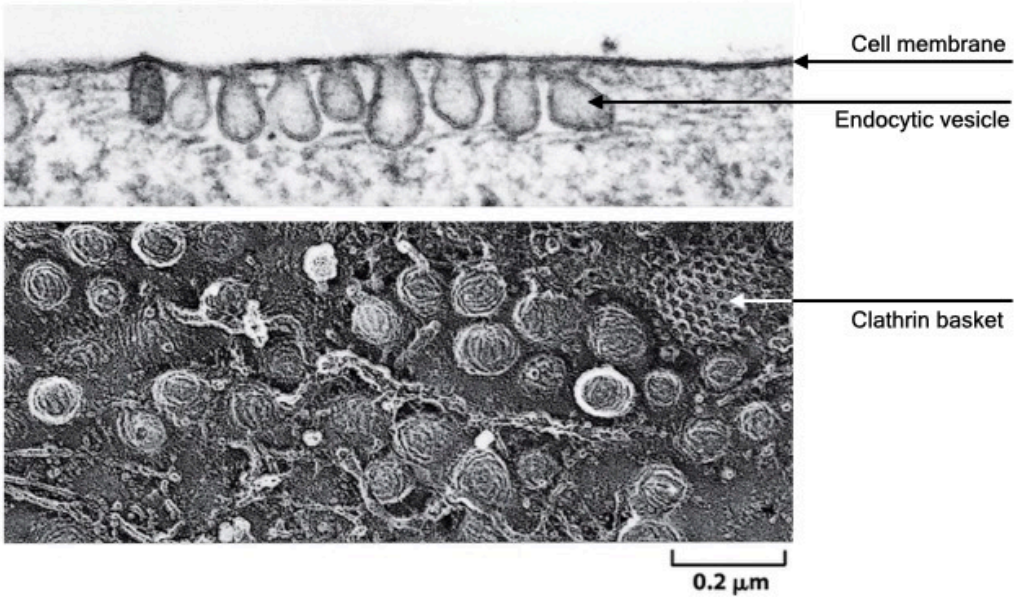
Endocytosis

Endocytosis comprises several pathways through which cells recycle proteins and lipids in the cell membrane and take up material from external environment. These pathways include pinocytosis, receptor-mediated endocytosis, and phagocytosis.

Pinocytosis

Pinocytosis is process in which cells pinch off a small portion of their cell membranes to form a vesicle. Through pinocytosis the cell removes 1 to 3% of plasma membrane per minute. Pinocytosis is critical for a cell's viability because over time proteins and lipids in the cell membrane are modified by oxidation or damaged by other factors. If this material was not removed, the function and structural integrity of the cell membrane would decline and potentially cause cell death. Cells use the secretory pathway to replace protein and lipid removed through pinocytosis. Pinocytosis appears to occur randomly along the surface of the cell membrane, so eventually most proteins and lipids are recycled.

Formation of a vesicle in pinocytosis is usually driven by a set of proteins called clathrin. Clathrin proteins form a basket-like coat on the cytoplasmic side of the cell membrane and facilitate formation and pinching off of a vesicle. Once formed, the vesicles are called clathrin-coated vesicles. Eventually, the clathrin coat falls off the vesicle, and the vesicle fuses with the lysosome where its digestive enzymes breakdown the protein and lipids in the vesicle.

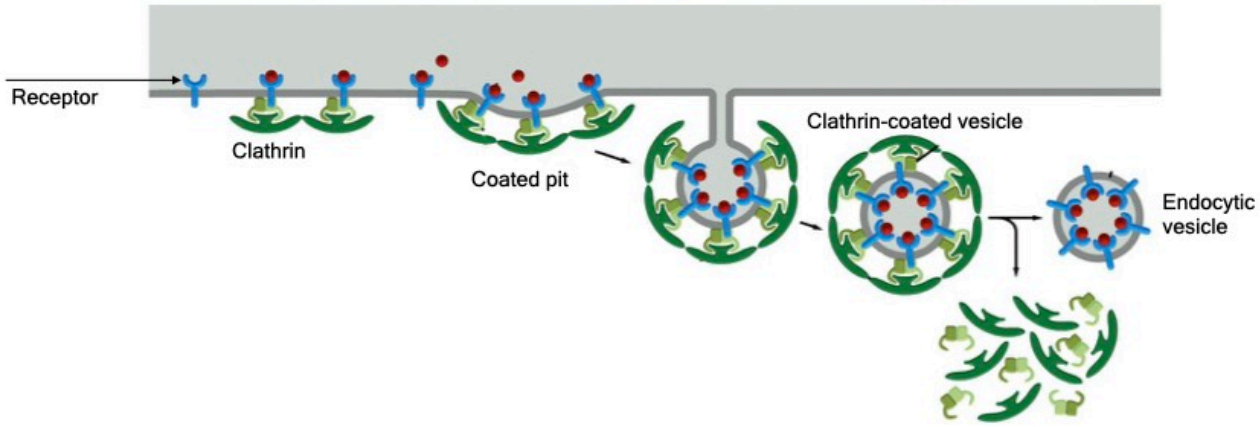


Pinocytosis ingests small amount of the cell membrane through formation of endocytic vesicles.

During pinocytosis, cells also take up small amounts of external fluid with the cell membrane, which allows them to sample the external environment.

Receptor-Mediated Endocytosis

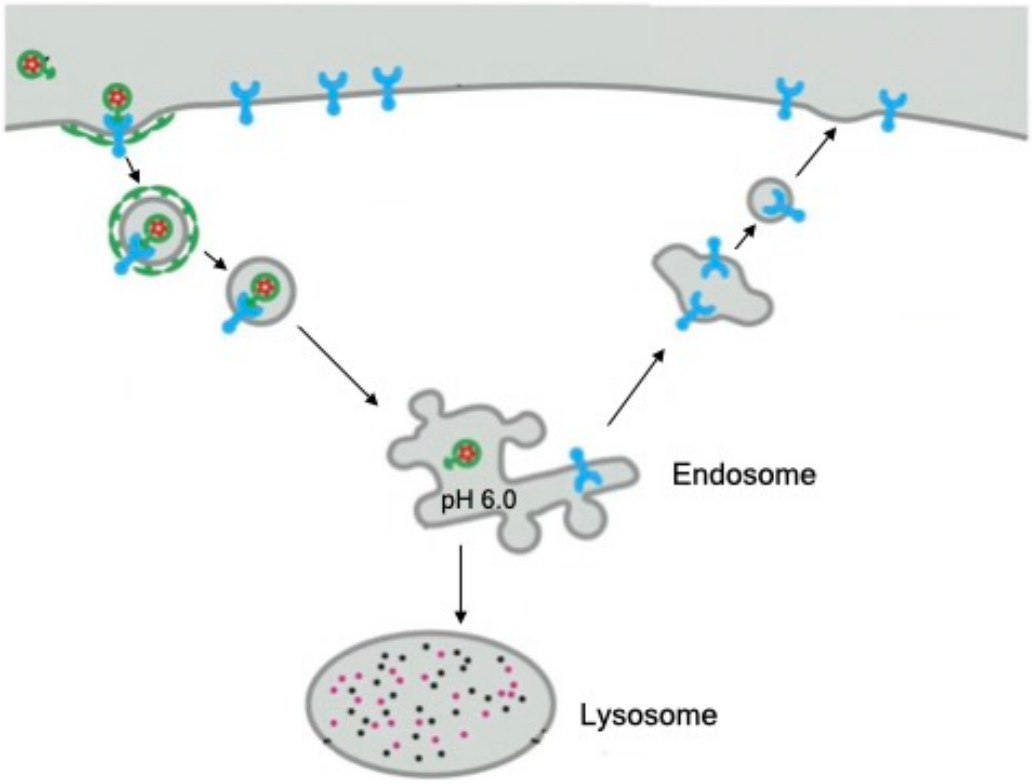
Most cells also have a mechanism to remove specific proteins from their cell membranes. Here, clathrin forms at specific sites on the cell membrane and recruits certain proteins into the endocytic vesicles. Cells mark proteins for endocytosis by modifying a domain that faces the cytoplasm. This modification is recognized by the clathrin machinery which form a clathrin coat near the location of modified proteins and recruit other modified proteins into the site. The endocytic vesicle that forms has a high concentration of modified protein.



Receptor-mediated endocytosis takes up specific proteins from the cell membrane.

Receptor-mediated endocytosis serves several functions. One is to take up nutrients that cells need to survive. Some receptors bind material that contain essential nutrients. Receptor-mediated endocytosis brings that material into the cell where it can be processed in the lysosome to release the nutrients. Receptor-mediated endocytosis also allows cells to regulate the amount of a specific protein in its cell membrane. This will be important when we discuss cell communication and how cells limit their response to a signal.

Similar to vesicles that form through pinocytosis, vesicles that form via receptor-mediated endocytosis are targeted to the lysosome. However, the cell also has the option of recycling receptors back to the cell membranes before the vesicle fuses with the lysosome. After the clathrin coat falls off the vesicle, the endocytic vesicle acidifies to dissociate the receptor from its cargo. At this point the vesicle is called an endosome. The receptor can be recycled from the endosome to the cell membrane and the cargo delivered to the lysosome.

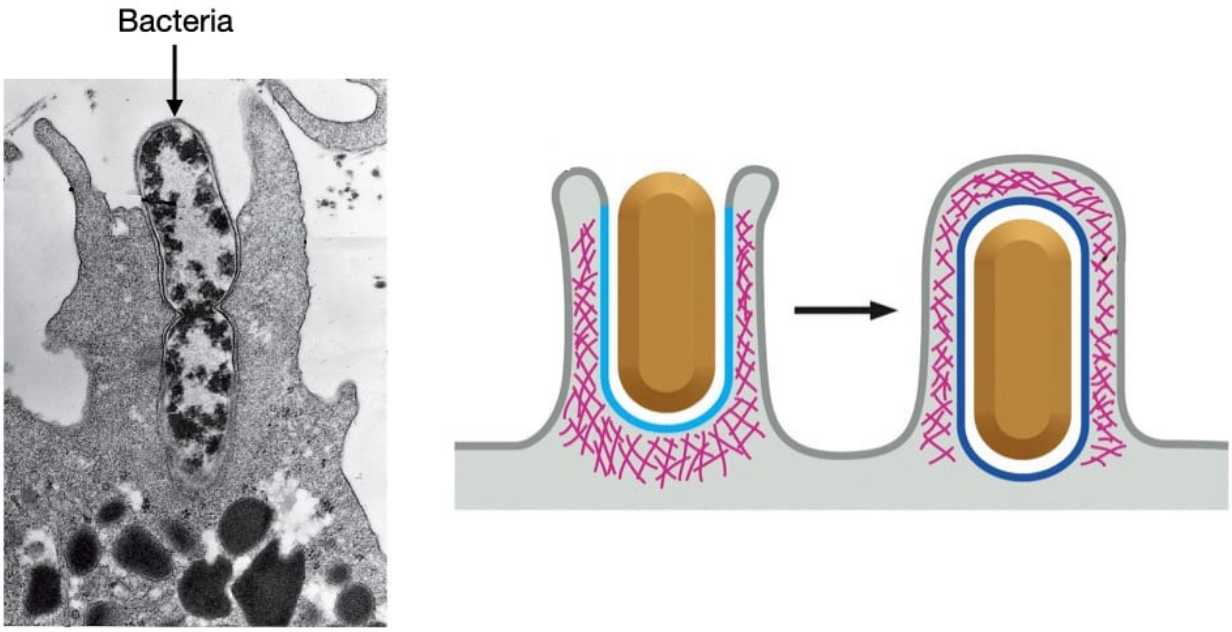


Recycling pathway returns receptors to cell membrane and delivers cargo to the lysosome.

Phagocytosis

Phagocytosis is a process through which certain cells engulf large objects, such as bacteria or cellular debris. Phagocytosis is usually found only in certain immune cells like macrophages and neutrophils. Phagocytosis is triggered when a macrophage or neutrophil binds a foreign object on

its cell membrane. The cell then pushes its cell membrane around object through an actin-dependent process to engulf the object. Once completely surrounded by cell membrane, the object will usually be delivered to the lysosome for degradation.



Phagocytosis engulfs large particles and microorganisms.