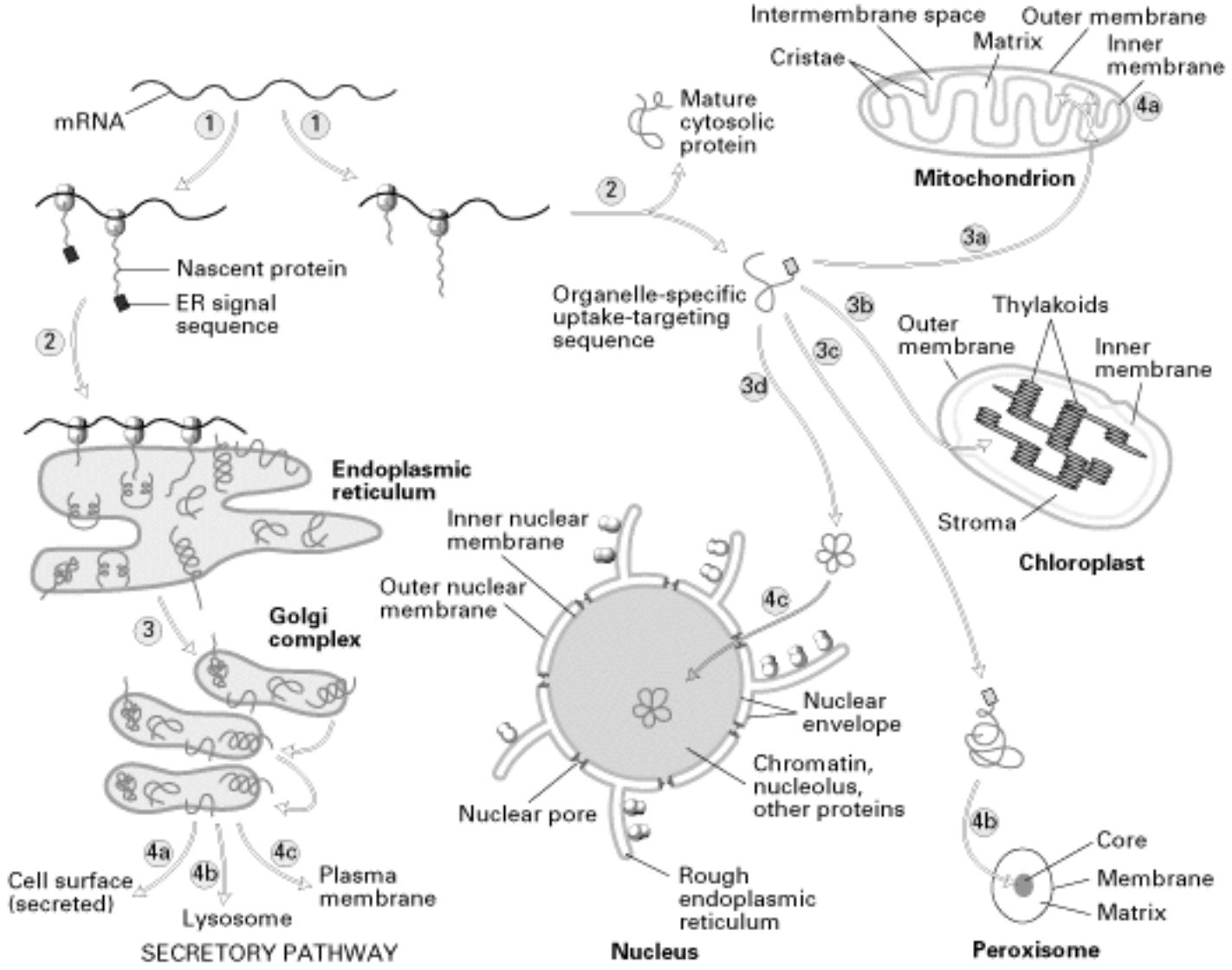
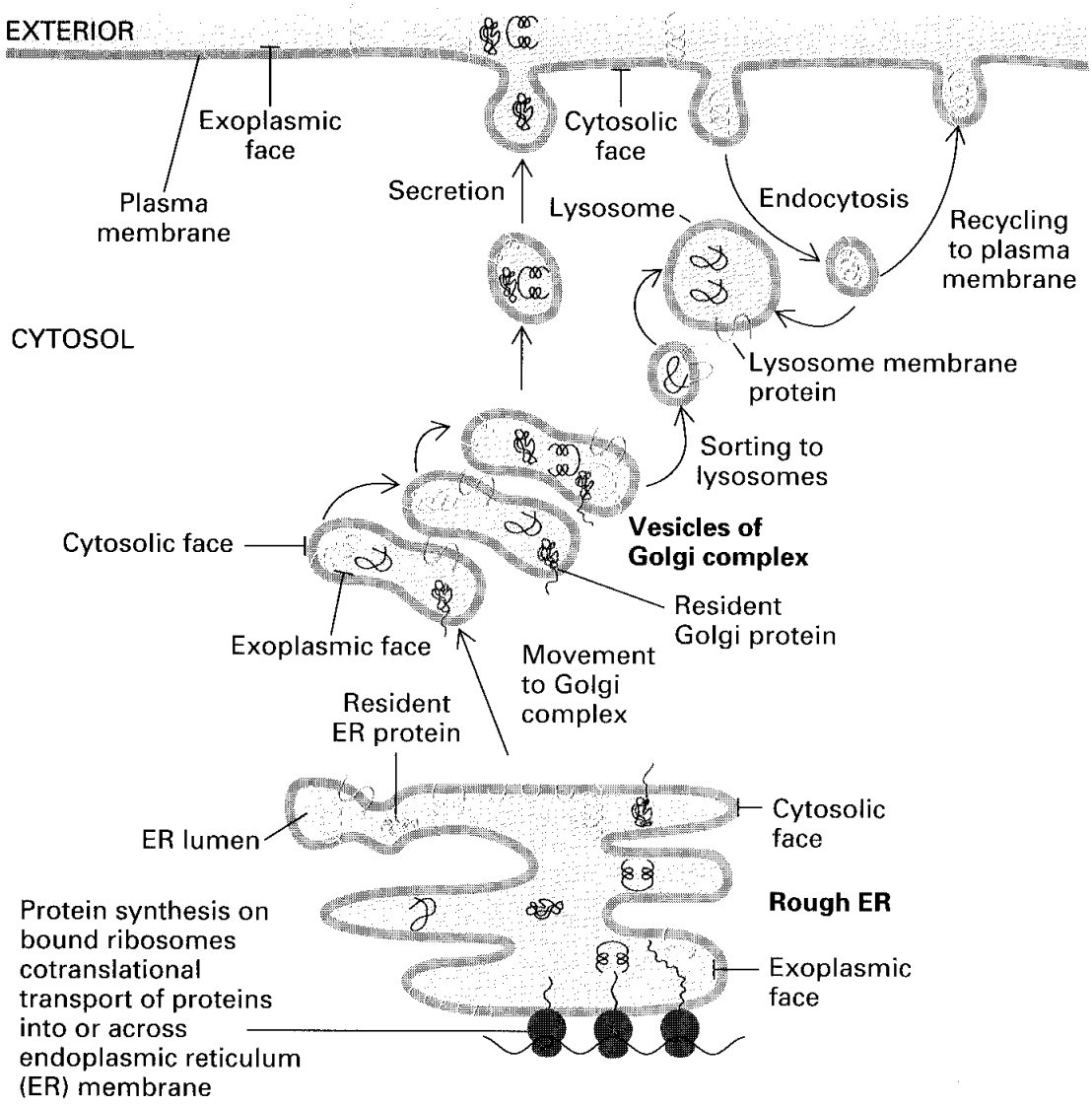
Protein Trafficking in the Secretory and Endocytic Pathways



The compartmentalization of eukaryotic cells has considerable functional advantages for the cell, but requires elaborate mechanisms to ensure that nascent proteins are correctly targeted to the appropriate compartment. This targeting occurs as the result of a series of binary (yes/no) decisions that commence at the time of synthesis of the nascent polypeptide. Proteins carry codes in their sequences that are read by targeting machinery at every stage of their voyage to their ultimate location.

Proteins may be targeted to the cytosol, mitochondria, peroxisomes or chloroplasts. These proteins (if encoded in the nucleus) are synthesized on free ribosomes. However, proteins destined for secretion, for the lumen of the ER, Golgi or lysosomes, or for the membrane of any of these organelles or the plasma membrane are synthesized on the membrane bound ribosomes of the rough ER. They are then targeted to the appropriate cellular compartment.

The Secretory Pathway



Topological equivalents

The interior of the ER, Golgi and secretory vesicles can be considered to be the topological equivalent of the outside of the cell. This topological equivalence is maintained throughout the secretory pathway, through the complicated series of budding and fusion events.

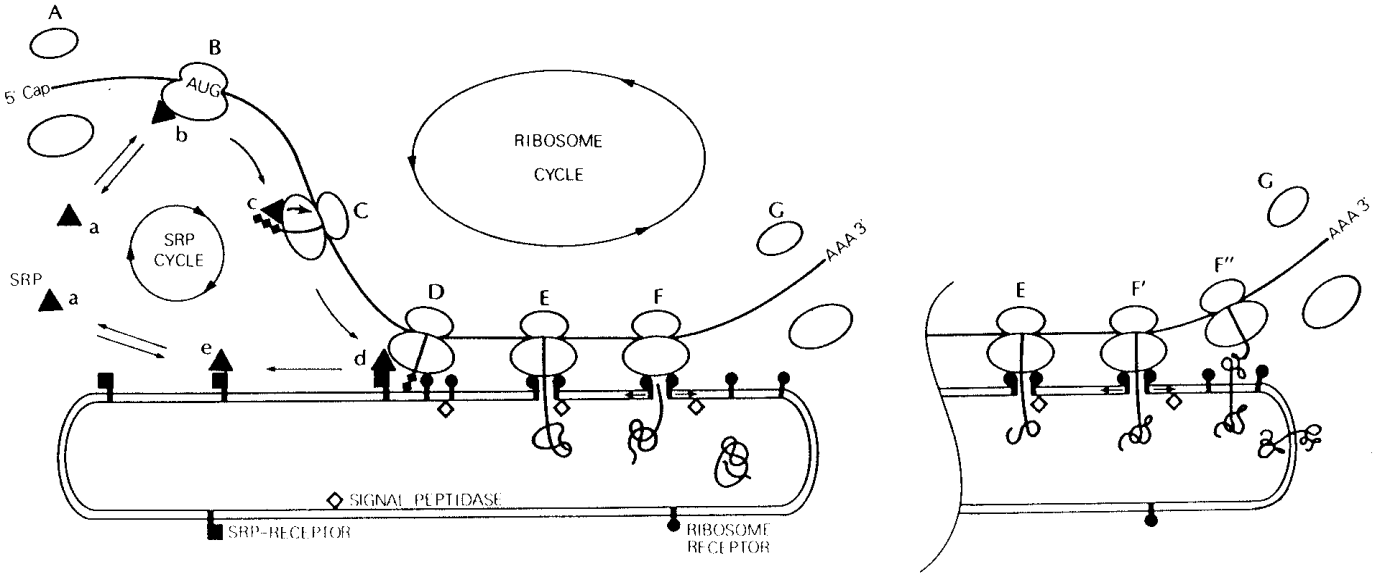
Vesicular Trafficking

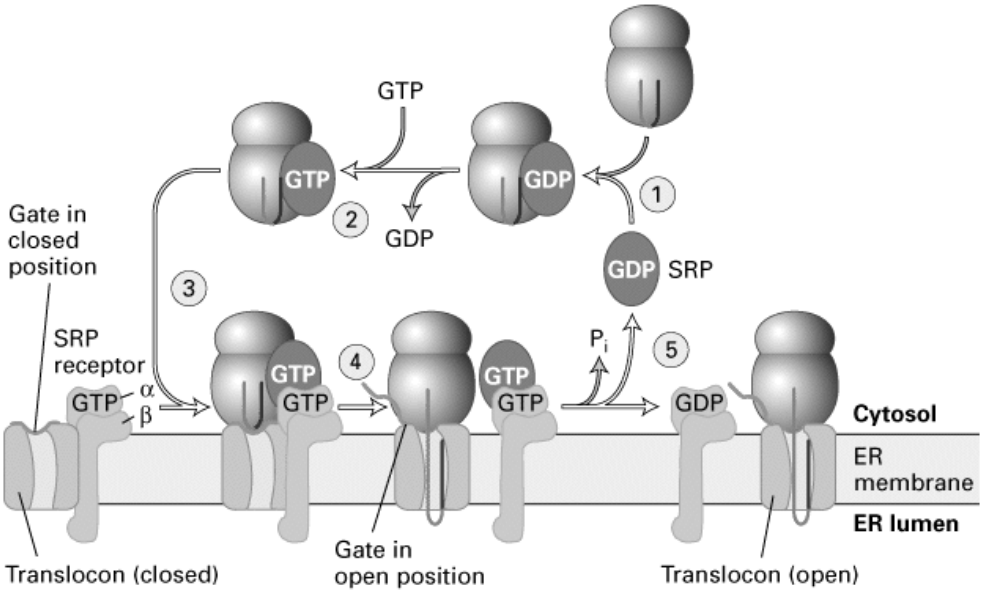
Proteins are generally believed to move between the compartments of the secretory pathway by budding and fusion of transport vesicles between successive compartments. This maintains the distinct internal environments of the compartments.

Co-Translational Targeting of Proteins

Nearly all proteins destined for any of the locations reached by the secretory pathway are targeted to the ER membrane as they are synthesized. Proteins are directed to the ER by a signal sequence. This is characteristically a stretch of 9 or more hydrophobic residues. The signal sequence is

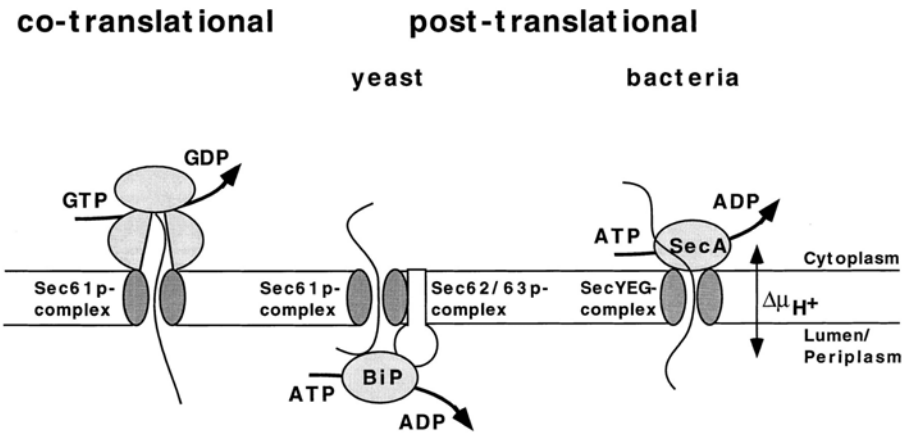
recognized by the signal recognition particle, a ribonucleoprotein complex.



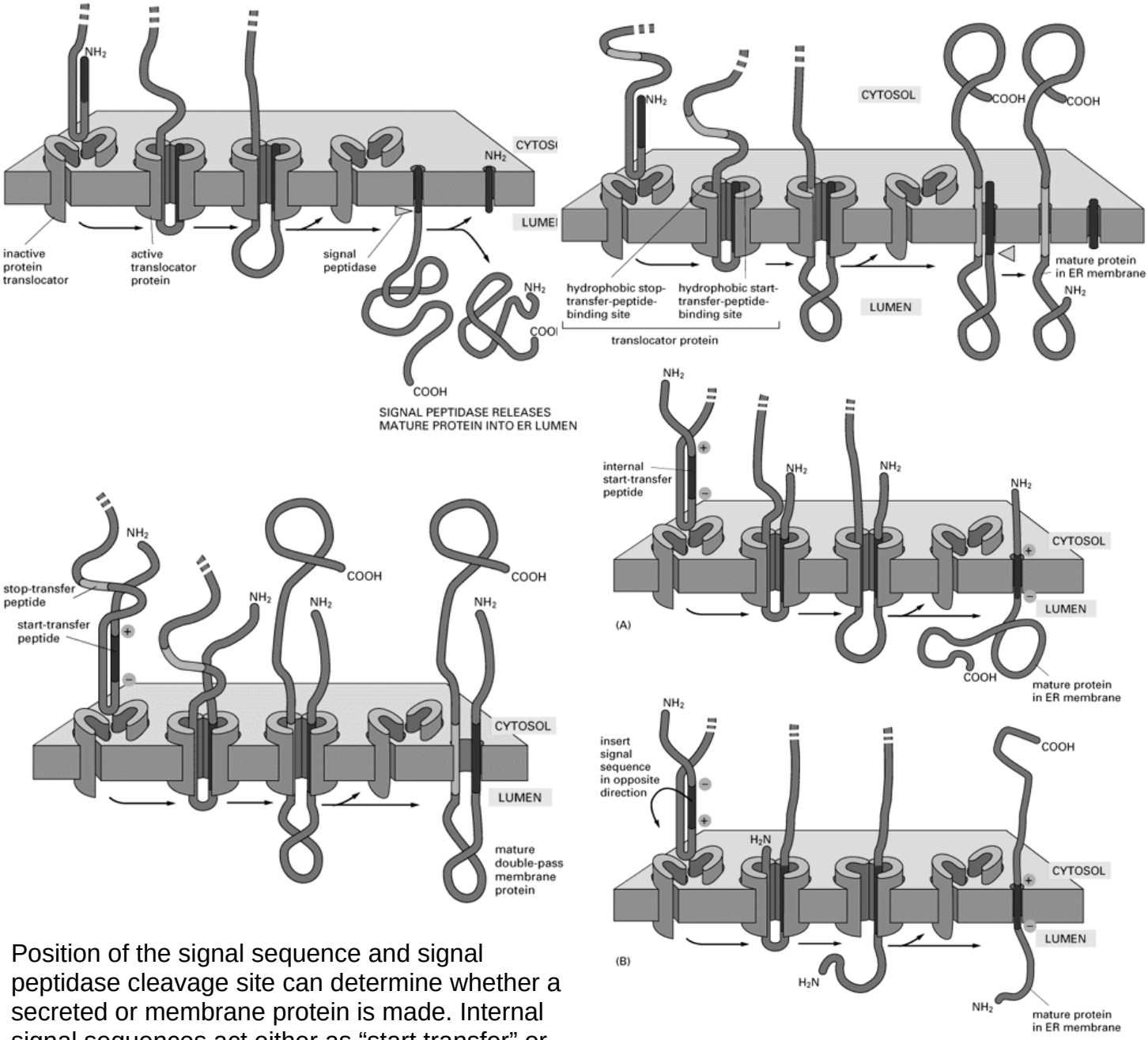


Binding of the ribosome and SRP complex to the SRP receptor initiates assembly of the **Sec61** complex, which acts as the translocator to allow passage of the polypeptide chain across the bilayer. GTPase activity associated with the SRP and the SRP receptor coordinates association and dissociation of the ribosome with opening and closing of the channel. This maintains the integrity of the barrier between the ER lumen and the cytosol.

A few proteins are post-translationally imported into the ER, by a mechanism that appears to be similar to that used for translocation across bacterial plasma membranes. This is more common in yeast, but rare in metazoans.

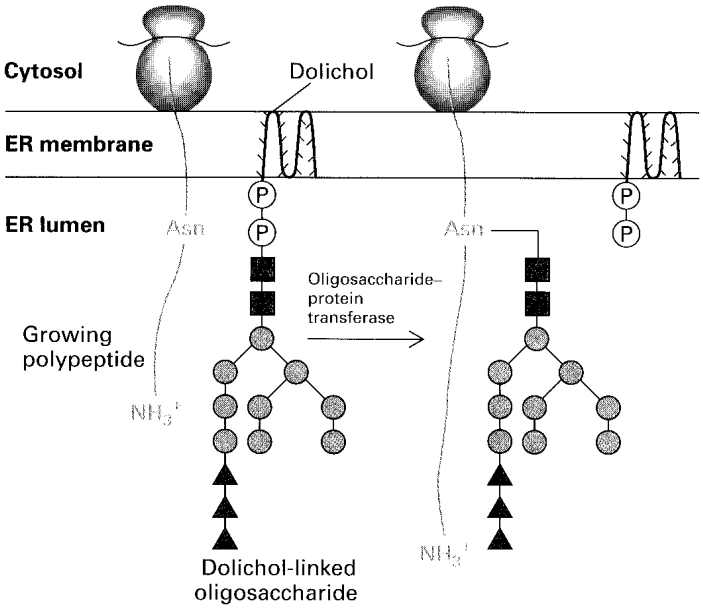


The ER signal sequence is cleaved off from most secreted proteins by a signal peptidase on the lumenal side of the ER membrane. However, signal sequences that occur within the polypeptide chain instead of at the N-terminus do not have peptidase cleavage sites and are never cleaved.



Position of the signal sequence and signal peptidase cleavage site can determine whether a secreted or membrane protein is made. Internal signal sequences act either as “start transfer” or “stop-transfer” signals depending on the order in which they occur in the sequence of the protein from the N-terminus. The first such sequence acts as a “start transfer” sequence, the next as a “stop transfer” sequence. The charge distribution on either side of the signal sequence determines the orientation with which it is inserted into the translocator.

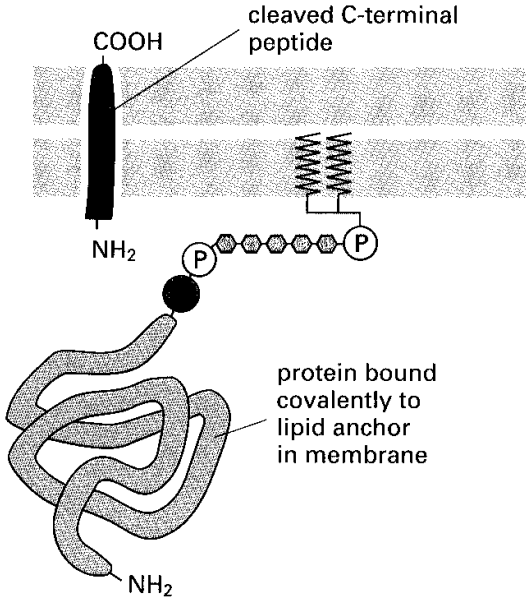
Protein import, oligomerization and folding are aided by chaperones (BiP) and protein disulphide isomerase within the ER lumen. Misfolded proteins are not permitted to pass further down the secretory pathway, but instead are degraded. Their continued association with chaperones prevents them from being packaged into transport vesicles destined for the Golgi. It has become clear recently that these proteins are degraded *in the cytosol* using the same ubiquitin-dependent machinery as cytosolic proteins. This means that polypeptides that are to be degraded have to be re-directed back through the translocation machinery. Thus, the translocation process can be reversed.



Post-Translational Modifications in the ER

Post-translational modification of secretory and membrane proteins begins in the ER and continues in the Golgi apparatus. ***N-linked glycosylation***

Attachment of a precursor oligosaccharide to asparagine residues occurs in the ER. This is the most common type of protein glycosylation. The precursor is transferred from a dolichol lipid moiety in the ER membrane by oligosaccharyl transferase. Immediately after the transfer, all three glucose residues and one mannose residue are removed. The precursor is further modified in the Golgi to produce the mature glycoprotein by trimming certain sugars and addition of others.

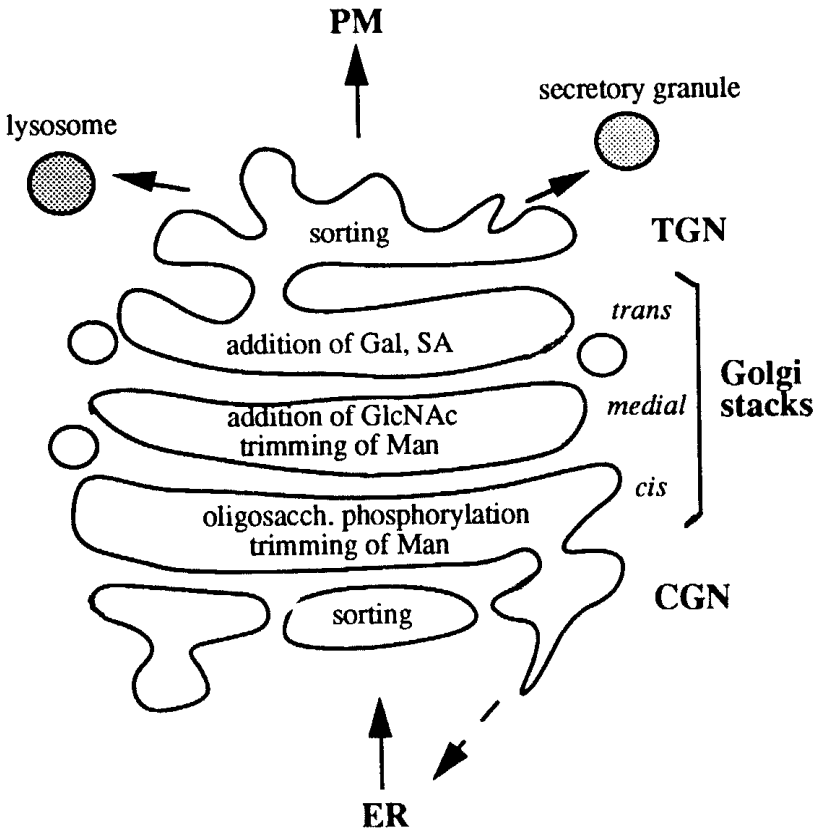


GPI-linkage

This is related to the prenylation of cytosolic proteins and directs otherwise soluble proteins to the membrane. A glucosylphosphatidylinositol anchor containing two fatty acids is added to the protein and at the same time the transmembrane segment is cut off. Ultimately the protein will be attached to the outside of the plasma membrane by its GPI anchor.

The Golgi Apparatus

The Golgi apparatus consists of an ordered series of compartments in which N-linked oligosaccharide chains are processed, O-linked oligosaccharides are added, and the proteoglycans of the



extracellular matrix are assembled. Distinct steps in the processing of N-linked oligosaccharides occur in the cis, medial and trans compartments of the Golgi. The Golgi also has important sorting functions.

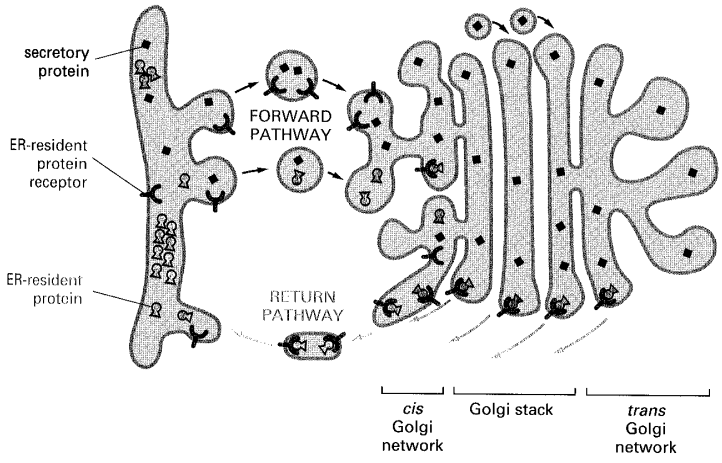
O-linked glycosylation

Sugar moieties are added sequentially (in contrast to N-linked glycosylation in which residues are added *en bloc*) to the hydroxyl groups of serine or threonine residues. An extreme example is the attachment of glycosaminoglycans via a link tetrasaccharide to serine residues of the core protein of proteoglycans.

Tagging Lysosomal Hydrolases

GlcNAc-phosphotransferases phosphorylate sugar residues on lysosomal hydrolases. These mannose-6-phosphate residues are recognized by a receptor that targets these proteins to the lysosome.

Sorting in the ER and Golgi



The default pathway

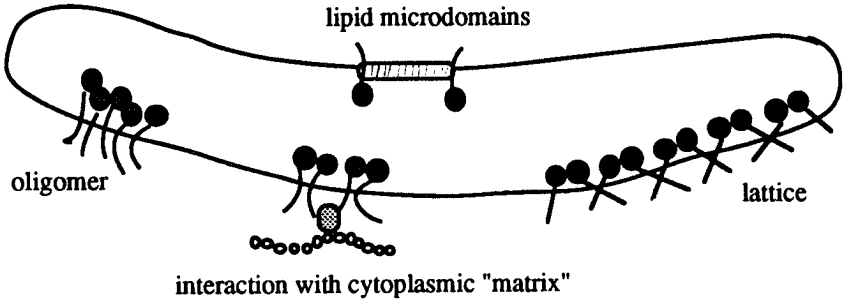
In non-polarized cells proteins synthesized on ER-bound ribosomes go to the cell surface by default, unless they are specifically directed to other compartments by special signals. Proteins that are to be retained in the ER or Golgi, or that are to be directed to specialized secretory vesicles, or to the lysosome must be sorted from those constitutively secreted. In addition, in polarized cells, signals direct proteins to specific domains of the cell surface.

ER resident proteins

Misfolded proteins are not allowed to leave the ER: they remain there bound to chaperones and are eventually degraded. Some soluble proteins are ER residents (e.g. BiP). These carry a sequence at their C-terminus (KDEL) that identifies them as ER residents. A KDEL receptor protein selectively returns ER resident proteins that escape to the cis Golgi network.

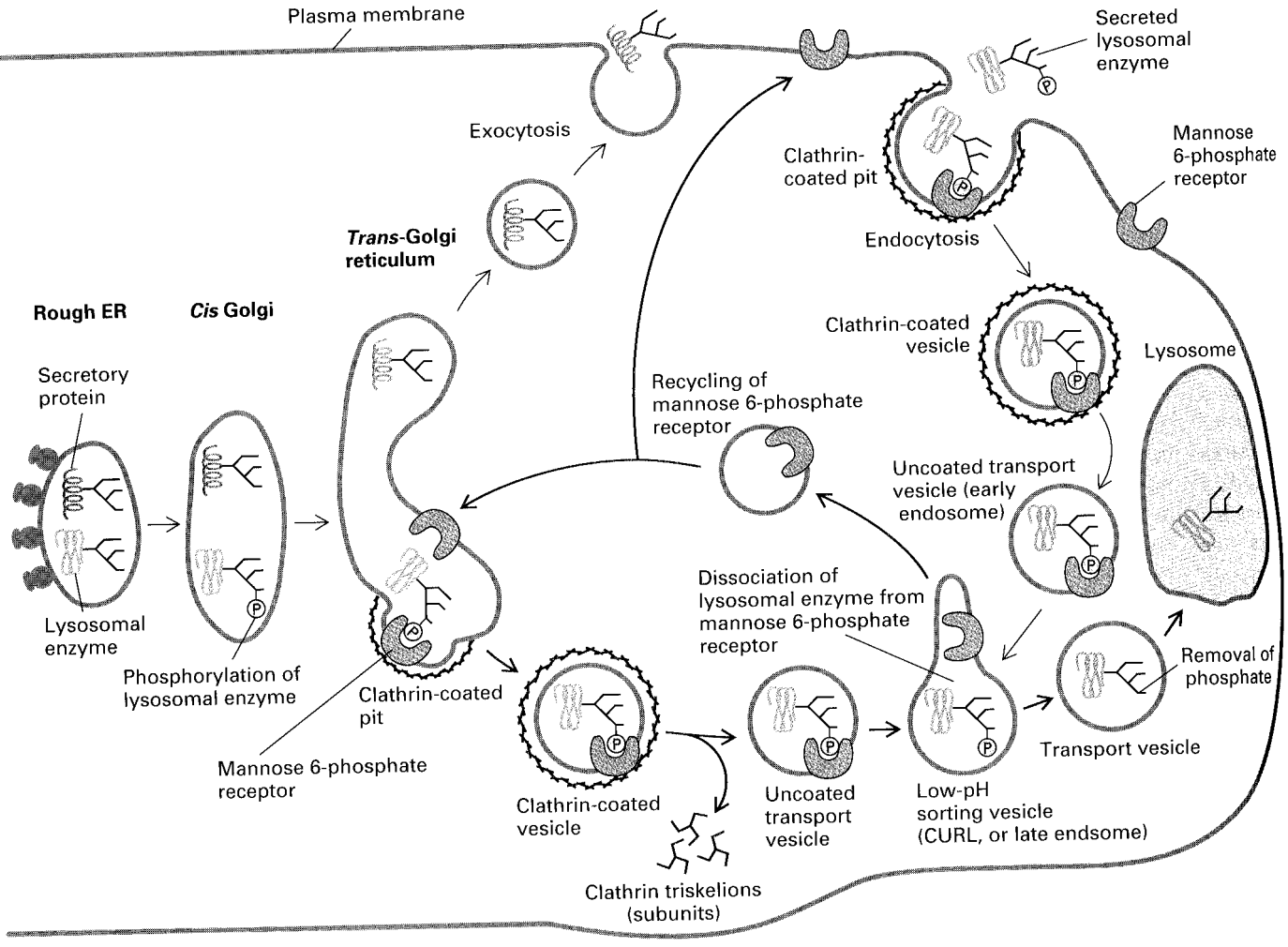
Retention of Golgi resident proteins

All known Golgi residents are membrane proteins. The signal for Golgi retention seems to be in the transmembrane domain of these proteins. These sequences may cause oligomerization of the proteins, thus preventing their incorporation in transport vesicles.



Sorting in the trans Golgi network

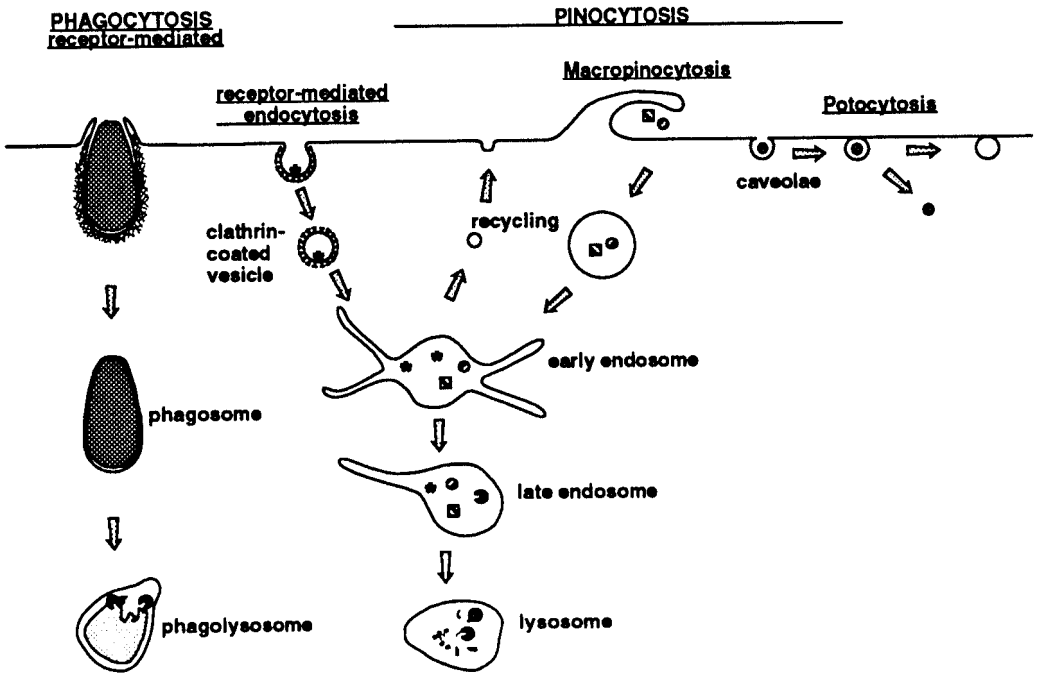
The trans Golgi network is an important sorting compartment within the secretory pathway. Here, lysosomal hydrolases bind to the mannose-6-phosphate receptor and are targeted to the lysosome. Proteins destined for regulated secretion (examples include digestive enzymes secreted in response to hormonal stimulation, or the hormones themselves) are packaged into vesicles that await a signal to discharge their contents. It is also a site for sorting proteins to the appropriate plasma membrane



domain in polarized epithelia. The figure above indicates the pathway by which lysosomal hydrolases are targeted to the lysosome. Note that the mannose-6-phosphate receptor must be retrieved from the lysosome to escape degradation.

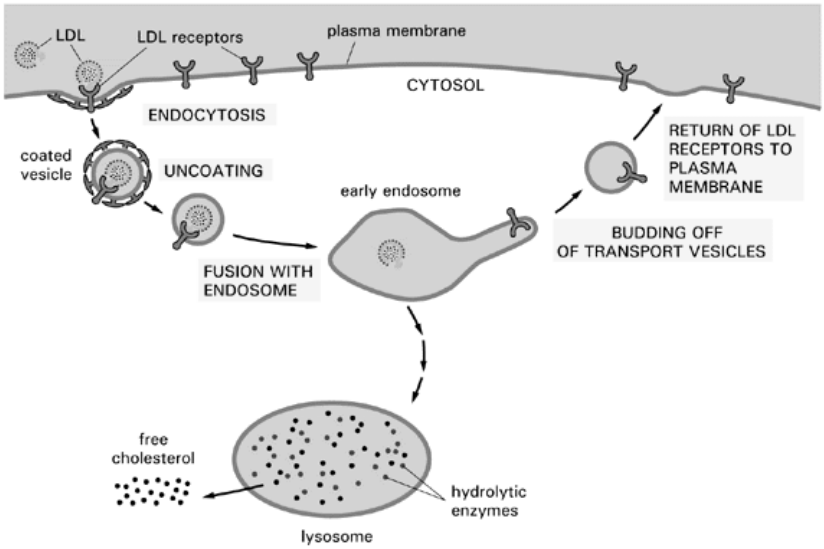
The Endocytic Pathway

Pinocytosis and Phagocytosis



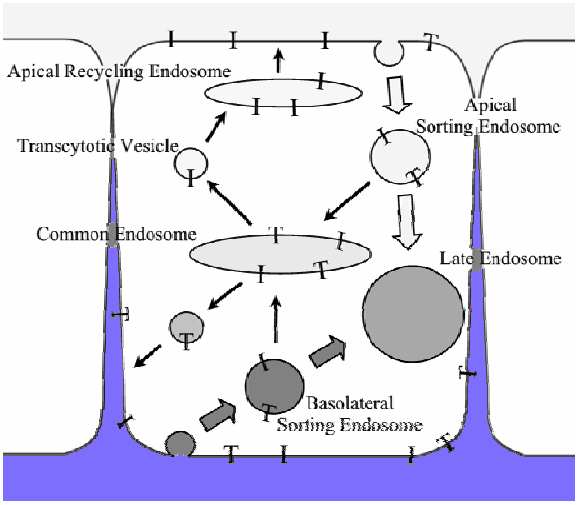
Pinocytosis is the constitutive mechanism of endocytosis found in all eukaryotic cells, as distinct from phagocytosis, which involves the uptake of large particles, such as whole cells, by specialized cells (many protozoa, and human macrophages, for example). Specific macromolecules are frequently taken up by specific receptors on the surface of the plasma membrane in the process of receptor-mediated endocytosis.

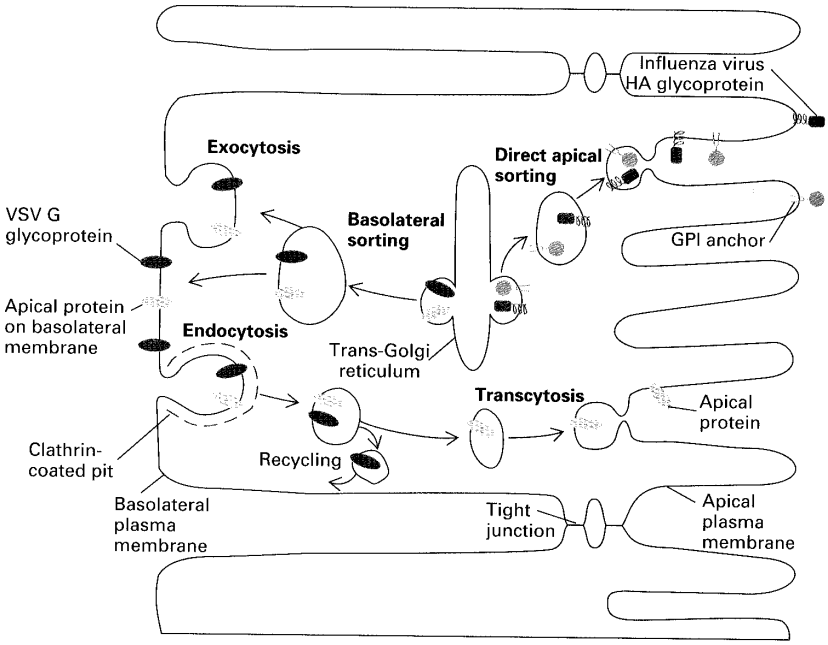
Molecules brought into the cell can be targeted to lysosomes for degradation, or recycled to the cell surface. Receptors and their ligands are sorted in the early or sorting endosome. The receptor-mediated uptake of LDL illustrates the pathway well. In the early or recycling endosome the ligand is induced to dissociate from the receptor because the pH is low. The ligand is segregated away from the receptors, which are segregated into tubulations that increase the surface:volume ratio.



Polarized Cells

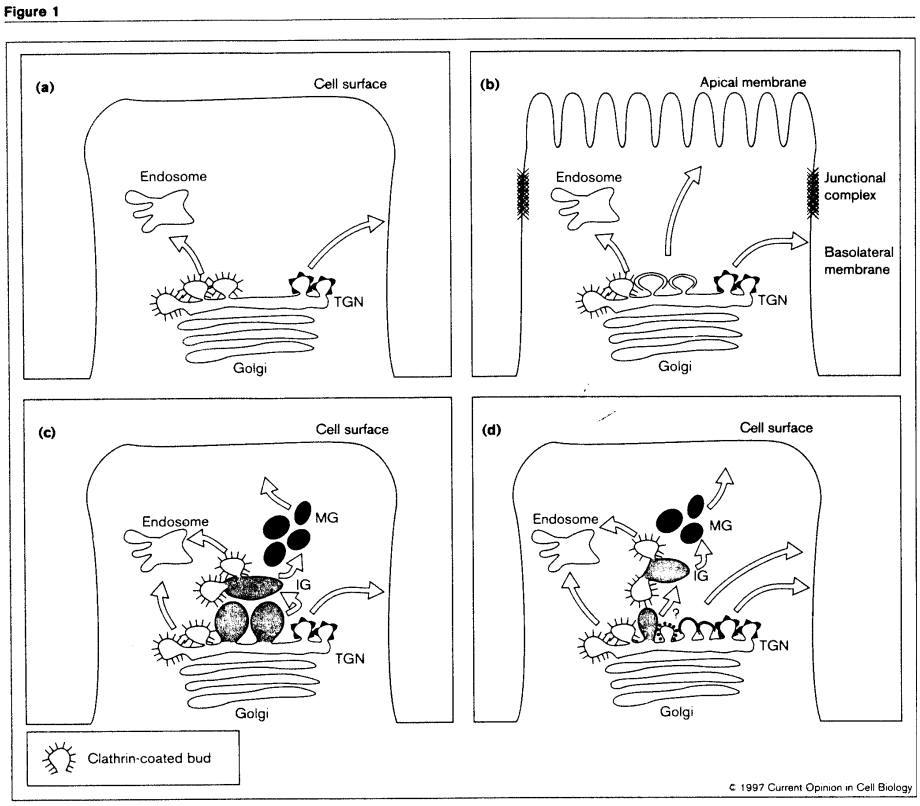
Most cell types in vertebrates are polarized. In polarized cells proteins must be sorted to the appropriate plasma membrane domain, either apical or basolateral. Sorting of proteins occurs in the





trans Golgi network. Receptors internalized from each domain initially enter an apical or basolateral compartment, but then very rapidly mix in a common compartment from which they must be sorted in order to recycle to the correct domain of the plasma membrane, so that receptors are only recycled to the proper domain (see above left).

Following synthesis, some receptors are delivered directly to the correct plasma membrane domain, while others are delivered randomly to the cell surface, and subsequently sorted in endosomes to the appropriate domain (transcytosis).



The figure (above right) illustrates an experiment in which a cell is simultaneously infected with two different viruses. The viral proteins move directly from the trans Golgi network to their appropriate domains, whereas a host cell apical membrane protein goes first to the basolateral surface and is then sorted in endosomes to the apical surface.

It is thought that in the TGN proteins may be segregated into different vesicle buds that are marked by having different coats on their cytoplasmic surface (left, see next lecture for more on coats). However, in most cell types it is likely that maintenance

of polarity is largely dependent on endocytic sorting.