# ER, Golgi and Vesicles :

Post-translational Processing and Vesicular Transport

Once a polypeptide has been translated and released from the ribosome, it may be ready for use, but often it must undergo post-translational processing in order to become fully functional. While many of these processes are carried out in both prokaryotes and eukaryotes, the presence of organelles provides the need as well as some of the mechanisms for eukaryote-specific modifications such as glycosylation and targeting.

Proteolytic Cleavage

The most common modification is proteolytic cleavage. Some of the pre-cleavage polypeptides are immediately cleaved, while others are stored as inactive precursors to form a pool of enzymes (or other kinds of proteins) that can be activated very quickly, on a timescale of seconds to minutes, as compared to having to go through transcription and translation, or even just translation. Interestingly, though methionine (Met) is universally the first amino acid of a newly synthesized polypeptide, many proteins have that methionine cleaved off (also true for some prokaryotic f-Met).

Figure 1. Proteolytic processing is necessary to make biologically active insulin. (A) The linear protein contains a signal sequence, which is cleaved after the protein enters the ER, an A chain, a B chain, and a C-peptide.   (B) Inside the ER, the proinsulin (insulin precursor) folds and disulfide bonds form between cysteines. (C) Finally, two cleavages release the C peptide, which leaves the A and B chains attached by the disulfide bonds. This is now active insulin.
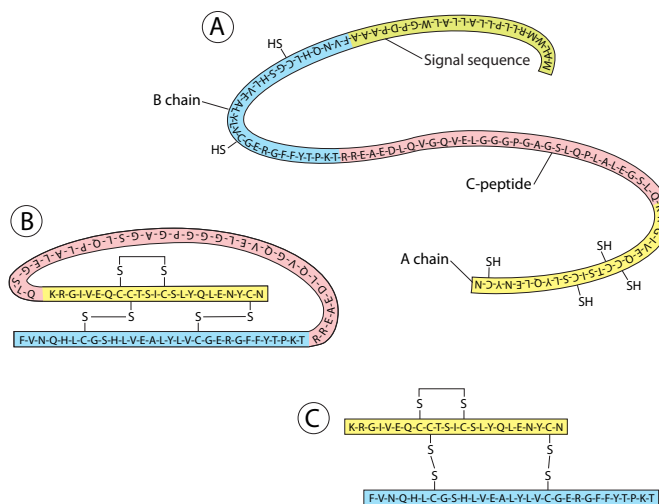
Activation of proteins by cleavage of precursors is a common theme: the precursor protein is termed a proprotein, and the peptide that is cleaved off of it to activate the protein is called the propeptide. Among the better known examples of proteins that are derived from proproteins are the hormone insulin, the cell death protein family of caspases, and the Alzheimer-associated neural protein β-amyloid. Insulin is an interesting example (fig. 1) in mammals: preproinsulin (inactive as a hormone) is first translated from the insulin mRNA. After a cleavage that removes an N-terminal sequence, proinsulin (still inactive) is generated. The proinsulin forms some internal disulfide bonds, and when the final proteolytic action occurs, a substantial chunk (called the C-peptide) is taken out of the middle of the proinsulin. Since the protein was internally disulfide bonded though, the two end pieces remain connected to become the active insulin hormone.

Another interesting protein processing example is that of collagen assembly (fig. 2). As you will read in chapter 13, collagen is a very large secreted protein that provides structure and shock absorbance for the extracellular matrix in animals. You can find it in skin, hooves, cartilage, and various connective tissues. An individual collagen protein is actually a twisted triple-helix of three subunits. The collagen subunits are made as procollagen, and propeptides are lopped off of both N- and C- termini to generate the final protein. However, they are not cleaved off until after the three subunits assemble around one another. In fact, collagen subunits that have already been processed do not assemble into triple-helical proteins. The propeptide sequences are clearly necessary for efficient assembly of the final protein complex.
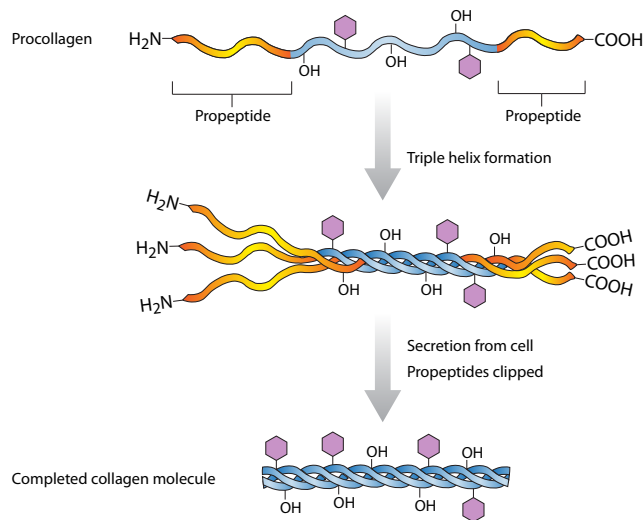

Figure 2. Processing and assembly of procollagen into collagen.

Protein Trafficking

The idea that propeptide sequences have important functions in protein maturation beyond just keeping them from being active is not exclusive to assembly. A major class of cleaved peptide sequences is signal peptides. Signal peptides direct the protein from the cytoplasm into a particular cellular compartment. In the case of prokaryotes, this essentially means the cell membrane, but for eukaryotes, there are specific signal peptides that can direct the protein to the nucleus, to the mitochondria, to the endoplasmic reticulum, and other intracellular organelles. The peptides are specifically recognized by receptors on the membranes of particular compartments, which then help to guide the insertion of the protein into or through the membrane. Almost all protein synthesis in eukaryotes is carried out in the cytoplasm (with the exception of a few proteins in the chloroplasts and mitochondria), so proteins found in any other compartment or embedded in any membrane must have been targeted and transported into that compartment by its signal sequence.

The nucleus is one such compartment, and examples of the proteins found within include DNA and RNA polymerases, transcription factors, and histones. These and other nuclear proteins have an N-terminal signal sequence known as the NLS, or nuclear localization signal. This is a well-studied pathway that involves a set of importin adapter proteins and the nuclear pore complex (fig. 3). Transport into the nucleus is particularly challenging because it has a double membrane (remember that it is contiguous with the endoplasmic reticulum membrane. Although there are other mechanisms for making proteins that are embedded in the nuclear membrane, the primary mechanism for import and export of large molecules into and out of the nucleus itself is the *nuclear pore complex*. The complex is very large and can be made of over 50 different proteins (nucleoporins, sometimes called nups). The nucleoporins are assembled into a large open octagonal pore through the nuclear membranes. As figure 3 indicates, there are antenna-like fibrils on the cytoplasmic face, and these help to guide proteins from their origin in the cytoplasm to the nuclear pore, and on the nuclear side there is a basket structure. Of course, not all proteins are allowed into the nucleus, and the mechanism for distinguishing appropriate targets is straightforward. The protein must bear a nuclear localization signal (NLS). While in the cytoplasm, an importin-α protein binds to the NLS of a nuclear protein, and also binds to an importin-β. The importin-β is recognized and bound by the nuclear pore complex. The details of the transport mechanism are murky, but phenylalanine-glycine repeats in the nucleoporin subunits (FG-nups) are thought to be involved.

Although this is primarily considered a eukaryotic process given that there are so many potential targets, prokaryotes do have membrane proteins (in fact, some 800 different ones in E. coli comprising ~20% of total protein), and they are positioned there with the aid of insertase enzymes such as YidC and complexes such as Sec translocase. The Sec translocase uses a signal recognition particle (SRP) much like that in eukaryotes, and will be discussed later in this chapter when the SRP is introduced. YidC, which has eukaryotic homologues (e.g. Oxa1 in mitochondria), is a 61 kDa transmembrane protein that is placed in the membrane through an SRP-Sec translocase mechanism. Once there, YidC interacts with nascent polypeptides (once they reach ~70 amino acids long) that have begun to interact with the lipids of the cell membrane, and pushes the protein into/through the membrane.

Figure 3. Transport through nuclear pore.
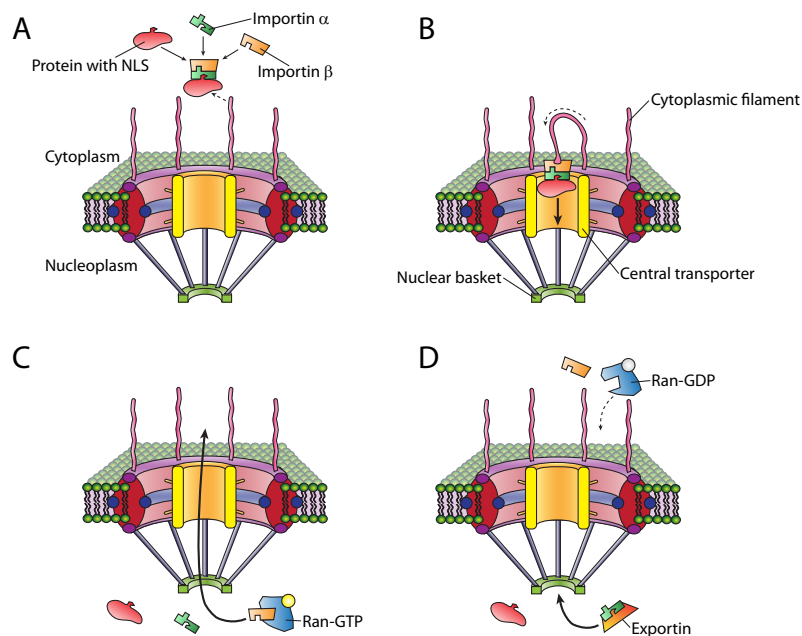
Once the nucleoprotein-importin aggregate is moved into the nucleus, Ran-GTP, a small GTPase, causes the aggregate to dissociate (fig. 3c). The imported protein is released in the nucleus. The importins are also released in the nucleus, but they are exported back out again to be reused with another protein targeted for the nucleus.

Export from the nucleus to the cytoplasm also occurs through the nuclear pore. The Ran-GTP is also a part of the export complex (fig. 3d), and in conjunction with an *exportin* protein and whatever is to be exported, is moved out of the nucleus via the nuclear pore. Once in the cytoplasm, the hydrolysis of GTP to GDP by Ran (activated by Ran-GAP, a cytoplasmic protein) provides the energy to dissociate the cargo (e.g. mRNA) from the exporting transport molecules. The Ran-GDP then binds to importins, re-enters the nucleus, and the GDP is exchanged for GTP.

The nuclear pore is the only transport complex that spans dual membrane layers, although there are coordinated pairs of transport complexes in double-membraned organelles such as mitochondria. The transport proteins in the outer mitochondrial membrane link with transport proteins in the inner mitochondrial membrane to move matrix-bound proteins (e.g. those involved in the TCA cycle) in from the cytoplasm. The complexes that move proteins across the outer membrane are made up of Tom (translocator outer membrane) family of proteins. Some of the proteins will stay em-

The mechanisms of small GTPase activation of other processes will be discussed again in more detail in later chapters (cytoskeleton, signaling). The key to understanding the mechanism is to remember that the GTPase hydrolyzes GTP to GDP, but still holds onto the GDP. Although the GTPase will hydrolyze GTP spontaneously, the GTPase-activating protein, GAP (or Ran-GAP in this case) greatly speeds the rate of hydrolysis. In order to cycle the system back to GTP, the GDP is not re-phosphorylated: it is *exchanged* for a new GTP. The exchange is greatly facilitated by the action of an accessory protein, the guanine nucleotide exchange factor (GEF), in this particular case, a Ran-GEF.

bedded in the outer membrane: they are processed by a SAM (sorting and assembly machinery) complex also embedded in the outer membrane). Meanwhile, others continue to the Tim (translocator inner membrane) proteins that move them across the inner membrane. As with the nuclear proteins, there is a consensus signal sequence on mitochondrial proteins that is bound by cytosolic chaperones that bring them to the Tom transporters. As shown in the table below, there are signal sequences/propeptides that target proteins to several other compartments.

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Nucleus              —PPKKKRKV—
ER entry             MMSFVSLLLVGILFWATEAEQLTKCEVFQ—
ER retention         —KDEL
Mito. Matrix         MLSLRQSIRFFKPATRTLCSSRYLL—
Peroxisome           —SKL
Peroxisome           —RLXXXXXHL
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Of particular importance for the rest of this chapter, is the sequence targeting proteins to the endoplasmic reticulum, and by extension, any proteins destined for the ER, the Golgi apparatus, the cell membrane, vesicles and vesicularly-derived compartments, and secretion out of the cell. Here, in addition to an N-terminal signal sequence, the position of secondary internal signal sequences (sometimes called signal patches) helps to determine the disposition of the protein as it enters the ER.

The initial insertion requires recognition of the signal sequence by *SRP*, the signal recognition protein. The SRP is a G-protein and exchanges its bound GDP for a GTP upon binding to a protein's signal sequence. The SRP with its attached protein then docks to a receptor (called the SRP receptor, astoundingly enough) embedded in the ER membrane and extending into the cytoplasm. The SRP usually binds as soon as the signal sequence is available, and when it does so, it arrests translation until it is docked to the ER membrane. Incidentally, this is the origin of the "rough" endoplasmic reticulum: the ribosomes studding the ER are attached to the ER cytoplasmic surface by the nascent polypeptide it is producing and an SRP. The SRP receptor can exist on its own or in association with a *translocon*, which is a bipartite translocation channel. The SRP receptor (SR) is also a GTPase, and is usually carrying a GDP molecule when unassociated. However, upon association with the translocon it exchanges its GDP for a GTP. These GTPs are important because when the SRP binds to the SR, both GTPase activities are activated and the resulting release of energy dissociates both from the translocon and the nascent polypeptide. This relieves the block on translation imposed by the SRP, and the new protein is pushed on through the translocon as it is being synthesized. Once the signal sequence has completely entered the lumen of the ER, it reveals a recognition site for *signal peptidase*, a hydrolytic enzyme that resides in the ER lumen and whose purpose is to snip off the signal peptide.

Prokaryotes also use an SRP homolog. In E. coli, the SRP is simple, made up of one protein subunit (Ffh) and a small 4.5S RNA. By comparison, some higher eukaryotes have an SRP comprised of six different proteins subunits and a 7S RNA. Similarly, there is a simple prokaryotic homologue to the SRP receptor, FtsY. An interesting difference is that FtsY generally does not interact with exported proteins, and appears to be necessary only for membrane-embedded proteins. Otherwise, there are many similarities in mechanism for SRP-based insertion of membrane proteins in eukaryotic and prokaryotic species, including GTP dependence, and completion of the mechanism by a translocase (SecYEG in E. coli).
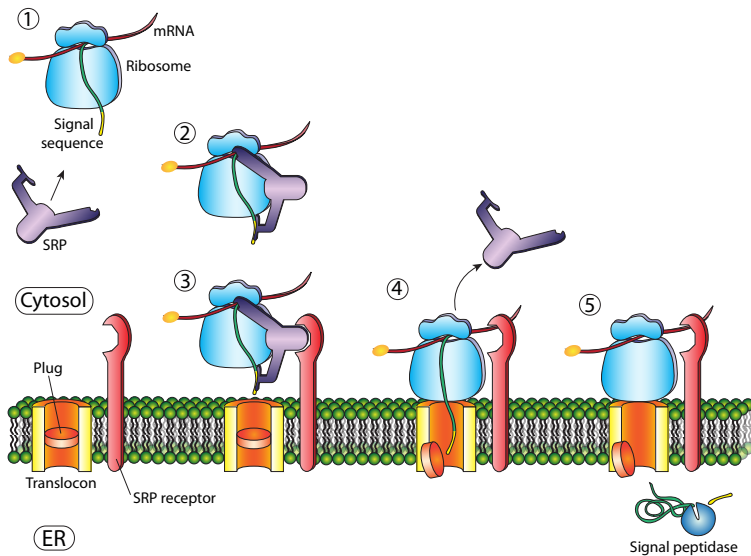
Figure 4. SRP and its receptor SR mediate movement of proteins through the ER membrane. The SRP recognizes the signal sequence and binds to it and the ribosome, temporarily arresting translation. The SRP-polypeptide-ribosome complex is bound by its receptor, SR, which positions the complex on a translocon. Once the ribosome and polypeptide are docked on the translocon, the SRP dissociates, and translation resumes, with the polypeptide moving through the translocon as it is being synthesized.

If that was the only signal sequence in the protein, the remainder of the protein is synthesized and pushed through the translocon and a soluble protein is deposited in the ER lumen, as shown in figure 4. What about proteins that are embedded in a membrane? Transmembrane proteins have internal signal sequences (sometimes called signal patches). Depending on their relative locations, they may be considered either *start*-transfer or *stop*-transfer sequence, where "transfer" refers to translocation of the peptide through the translocon. This is easiest to understand by referring to fig. 5. If

Figure 5. Single-pass transmembrane protein insertion. (1) the signal sequence has allowed the ribosome to dock on a translocon and newly made polypeptide is threaded through until the stop-transfer sequence. (2) The hydrophobic stop transfer sequence gets "stuck" in the membrane, forcing the rest of the polypeptide to stay in the cytoplasm as it is translated.
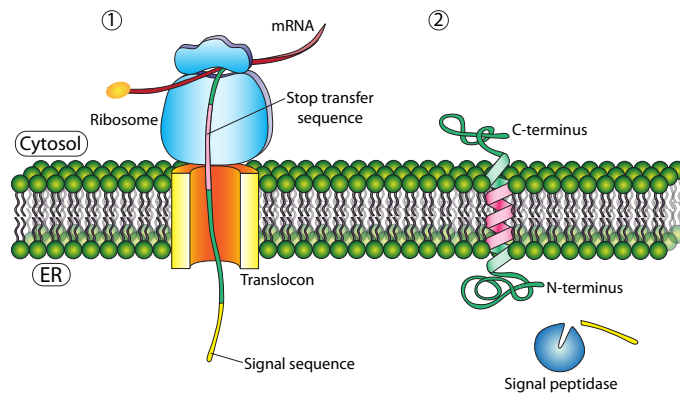
there is a significant stretch of mostly-uninterrupted hydrophobic residues, it would be considered a stop-transfer signal, as that part of the protein can get stuck in the translocon (and subsequently the ER membrane) forcing the remainder of the protein to remain outside the ER. This would generate a protein that inserts into the membrane once, with its N-terminus in the ER lumen and the C-terminus in the cytoplasm. In a multi-pass transmembrane protein, there could be several start- and stop- transfer hydrophobic signal patches. Building on the single-pass example, if there was another
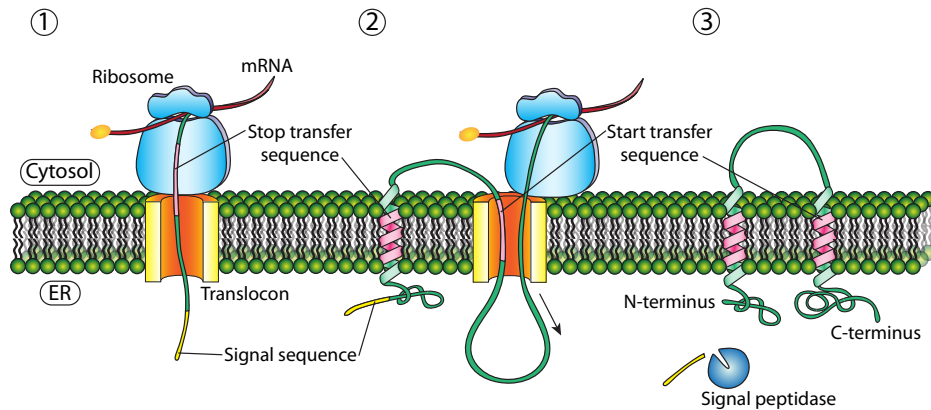

Figure 6. Insertion of 2-pass transmembrane protein.

signal patch after the stop-transfer sequence, it would act as a start-transfer sequence, attaching to a translocon and allowing the remainder of the protein to be moved into the ER. This results in a protein with both N- and C- termini in the ER lumen, passing through the ER membrane twice, and with a cytoplasmic loop sticking out. Of course, the N-terminus could be on the other side. For a cytoplasmic N-terminus, the protein cannot have an N-terminal signal sequence (fig. 7). It has an internal signal patch instead. It plays essentially the same role, but the orientation of the patch means that the N-terminal stays cytoplasmic. The polypeptide translated after the patch is fed into the ER. And just as in the last example, multiple stop- and start-sequences can reinsert the protein in the membrane and change the facing of the next portion.
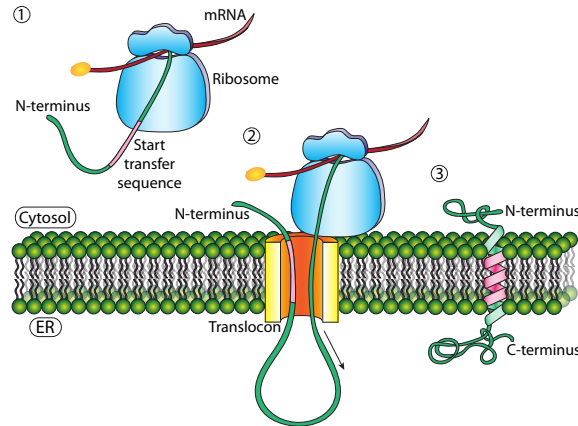

Figure 7. Insertion of a single-pass protein with N-terminus in cytoplasm uses a signal patch but no N-terminal signal.

Protein Folding in the ER

The ER lumen plays four major protein processing roles: folding/refolding of the polypeptide, glycosylation of the protein, assembly of multi-subunit proteins, and packaging of proteins into vesicles. Refolding of proteins is an important process because the initial folding patterns as the polypeptide is still being translated and unfinished may not be the optimal folding pattern once the entire protein is available. This is true not just of H-bonds, but of the more permanent (i.e. covalent) disulfide bonds as well. Looking at the hypothetical example polypeptide, the secondary structure of the N-terminal half may lead to the formation of a stable disulfide bond between the first cysteine and the second cysteine, but in the context of the whole protein, a more stable disulfide bond might be formed between cysteine 1 and cysteine 4. The exchange of disulfide bonding targets is catalyzed by protein disulfide isomerase (PDI). The internal redox environment of the endoplasmic reticulum, is significantly more oxidative than that in the cytoplasm. This is largely determined by glutathione, which is found in a 30:1 GSH:GSSG ratio or higher in the cytoplasm but at nearly 1:1 ratio in the ER lumen. This oxidative environment is also conducive to the disulfide remodeling. It should be noted that PDI does not choose the "correct" bonding partners. It simply moves the existing disulfide bonds to a more energetically stable arrangement. As the rest of the polypeptide continues to refold, breaking and making H-bonds quickly, new potential disulfide bond partners may move near one another and PDI can again attempt to rearrange the disulfide bonding pattern if the resulting pattern is more thermodynamically stable.
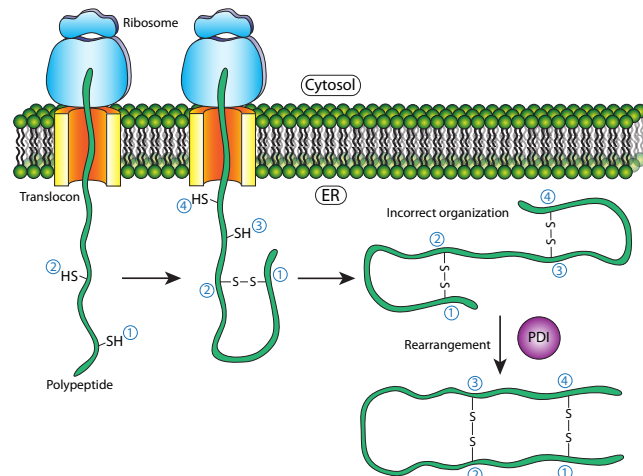

Figure 8. Protein Disulfide Isomerase rearranges disulfide bonds.

The assembly of multisubunit proteins and the refolding of polypeptides are similar in their use of chaperone proteins that help prevent premature folding, sequestering parts of the protein from H-bonding interaction until the full protein is in the ER lumen. This mechanism simply makes finding the thermodynamically optimal conformation easier by preventing the formation of some potential suboptimal conformations. These chaperone proteins bind to the new proteins as they enter the lumen through
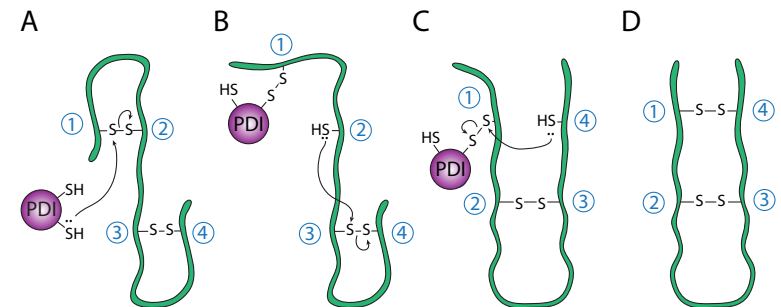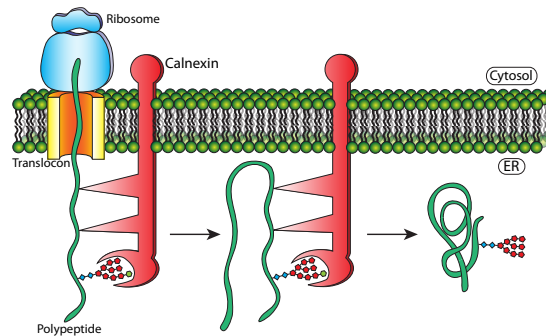

Figure 9. Protein Disulfide Isomerase. This enzyme uses a sulfhydryl group of a cysteine residue as temporary bonding partner in order to break disulfide bonds on the target protein and allow for new ones to form. Note that the formation of a new bond is not directed by PDI, but is instead a stochastic process in which a stronger binding partner displaces the PDI —SH.

Figure 10. Protein folding is optimized in the ER. Proteins such as calnexin can temporarily bind to nascent polypeptides, preventing them from forming secondary structures from incomplete information, releasing the protein for folding once the entire polypeptide has been translated.

Chaperone proteins can also be found in prokaryotes, archaea, and in the cytoplasm of eukaryotes. These are somewhat similar to each other, and function somewhat differently than the types of folding proteins found in the ER lumen. They are referred to generally as chaperonins, and the best characterized is the GroEL/GroES complex in E. coli. As the structure in figure 11 indicates, it is similar in shape to the proteasome, although with a completely different function. GroEL is made up of two stacked rings, each composed of 7 subunits, with a large central cavity and a large area of hydrophobic residues at its opening. GroES is also composed of 7 subunits, and acts as a cap on one end of the GroEL. However, GroES only caps GroEL in the presence of ATP. Upon hydrolysis of the ATP, the chaperonins undergo major concerted conformational changes that impinge on the protein inside, causing refolding, and then the GroES dissociates and the protein is released back into the cytosol.

the translocon and in addition to simply preventing incorrect bonds that would have to be broken, they also prevent premature interaction of multiple polypeptides with one another. This can be a problem because prior to the proper folding that would normally hide such domains within the protein, the immature polypeptides may have interaction domains exposed, leading to indiscriminate binding, and potentially precipitation of insoluble protein aggregates.

N-linked Protein Glycosylation Begins in the ER

Glycosylation is an important modification to eukaryotic proteins because the added sugar residues are often used as molecular flags or recognition signals to other cells than come in contact with them. There are two types of protein glycosylation, both of which require import of the target polypeptide into the ER. N-linked glycosylation actually begins in the endoplasmic reticulum, but O-linked glycosylation does not occur until the polypeptide has been transported into the Golgi apparatus. Therefore, it is also the case that N-linked glycosylation can (and is) usually beginning as a co-translational mechanism, whereas O-linked glycosylation must be occurring post-translationally. Other major differences in the two types of glycosylation are (1) N-linked glycosylation occurs on asparagine (N) residues within an N-X-S or N-X-T sequence (X is any amino acid other than P or D) while O-linked glycosylation occurs on the side chain hydroxyl oxygen of either serine or threonine residues determined not by surrounding sequence, but by secondary and tertiary structure; (2) N-linked glycosylation begins with a "tree" of 14 specific sugar residues that is then pruned and remodeled, but remains fairly large, while O-linked glycosylation is based on sequential addition of individual sugars, and does not usually extend beyond a few residues.

Technically, N-glycosylation begins before a protein is even being translated, as the dolichol pyrophosphate oligosaccharide (i.e. the sugar "tree" - not an official term, by the way) is synthesized in the ER (fig. 12) without being triggered by translation or
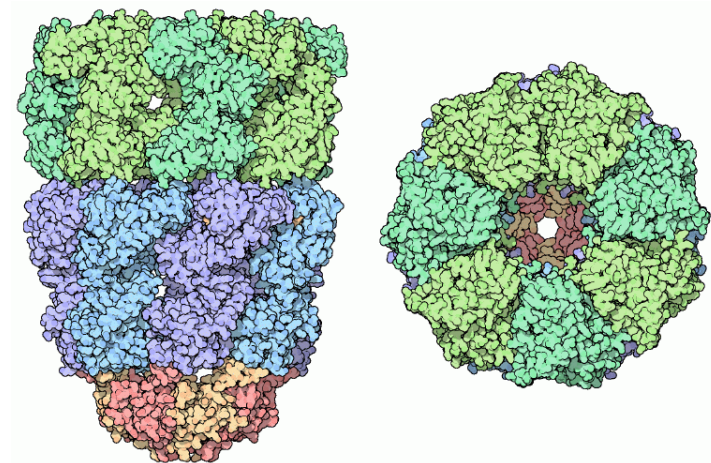

Figure 11. GroEL/GroES complex. The two heptameric rings of GroEL are shown in green and blue/purple. The GroES heptamer (red/yellow) caps the GroEL complex in the presence of ATP. Illustration by D.S. Goodsell, 2002.
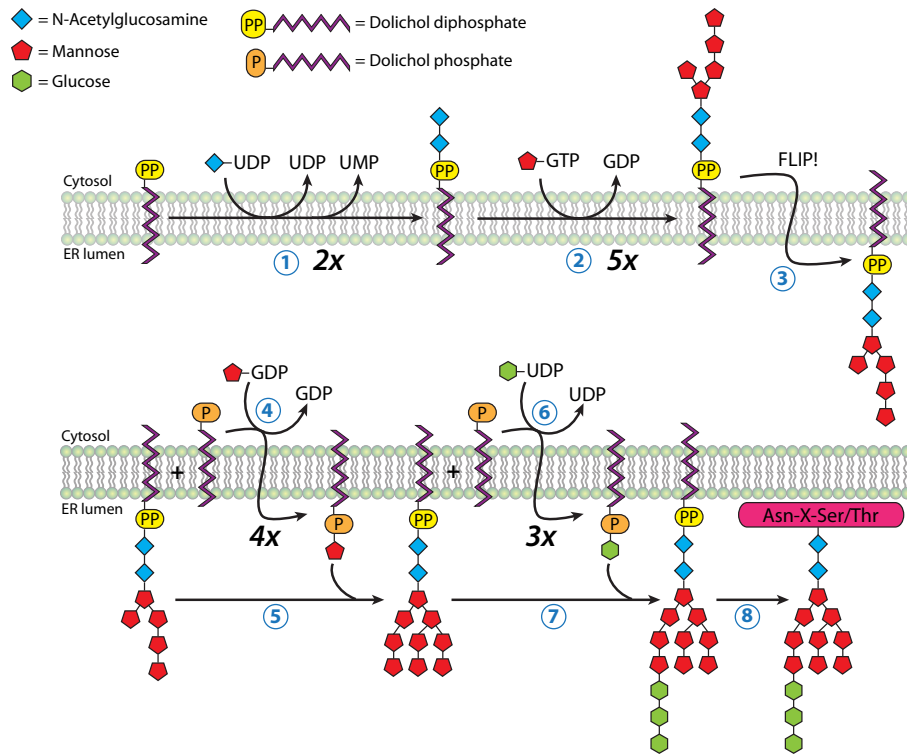
Figure 12. Formation of N-glycosylation "sugar tree" and attachment to protein. Each step is catalyzed by a glycosyltransferase. Note that the sugar substrates are sugar nucleotides, not isolated sugar molecules.

protein entry. Dolichol is a long-chain hydrocarbon [between 14-24 isoprene units of 4+1 carbons] found primarily in the ER membrane, and serves as a temporary anchor for the N-glycosylation oligosaccharide as it is being synthesized and as it waits for an appropriate protein to glycosylate. The oligosaccharide synthesis begins with the addition of two N-acetylglucosamine residues to the pyrophosphate linker, followed by a mannose. From this mannose, the oligosaccharide branches, with one branch receiving three more mannose residues and the other receiving one. So far, all of these additions to the oligosaccharide have been taking place in the cytoplasm. Now the glycolipid is *flipped* inwards to the ER lumen! Once in the lumen, four more mannoses are added, and finally three glucose residues top off the structure.

The enzymes that accomplish the glycosylation are glycosyltransferases specific for both the added sugar residue and the target oligosaccharide. The sugars used by the enzymes are not simply the sugar, but nucleotide sugars - usually a sugar linked to a nucleoside diphosphate, for example, uracil diphosphate glucose (UDP-glucose) or GDP-mannose.

Not all nucleosides are used for this process: sugars have only been found linked to UDP, GDP, and CMP. UDP is the most versatile, binding N-acetylgalactosamine (GalNAc), N-acetylglucosamine (GlcNAc), N-acetylmuramic acid, galactose, glucose, glucuronic acid, and xylose. GDP is used for mannose and fucose, while CMP is only used for sialic acid.

The N-linked oligosaccharide has two physiological roles: it acts as the base for further glycosylation, and it is used as a marker for error-checking of protein folding by the calnexin-calreticulin system (fig. 13). Once the oligosaccharide is attached to the new polypeptide, the process of further glycosylation begins with the action of a glucosidase and that removes two of the glucoses. The last glucose is necessary to help the glycoprotein dock with either calnexin or calreticulin (fig.13, step 1 or 4), which are very similar proteins that have a slow glucosidase activity and associate with a protein disulfide isomerase-like activity. The major difference is that calreticulin is soluble in the ER lumen while calnexin is bound to the ER membrane. Both temporarily hold onto the glycoprotein giving it time to (re)fold and possibly rearrange disulfide bonds, then it removes the glucose, allowing the glycoprotein to continue on its way. Importantly, if the glycoprotein has not been completely folded (step 2a), the enzyme UDP-glucose:glycoprotein glucosyltransferase (GT) recognizes it and adds back the glucose residue (step 3), forcing it to go through the calreticulin/calnexin cycle again in hopes of folding correctly this time. If it has been folded correctly (step 2b), it can be recognized by ER-α-1,2-mannosidase, which removes a mannose, completing the glycosylation modifications in the ER.

The protein disulfie isomerase-like activity comes from ERp57, which is technically a thiol oxidoreductase, but is functionally similar to PDI.
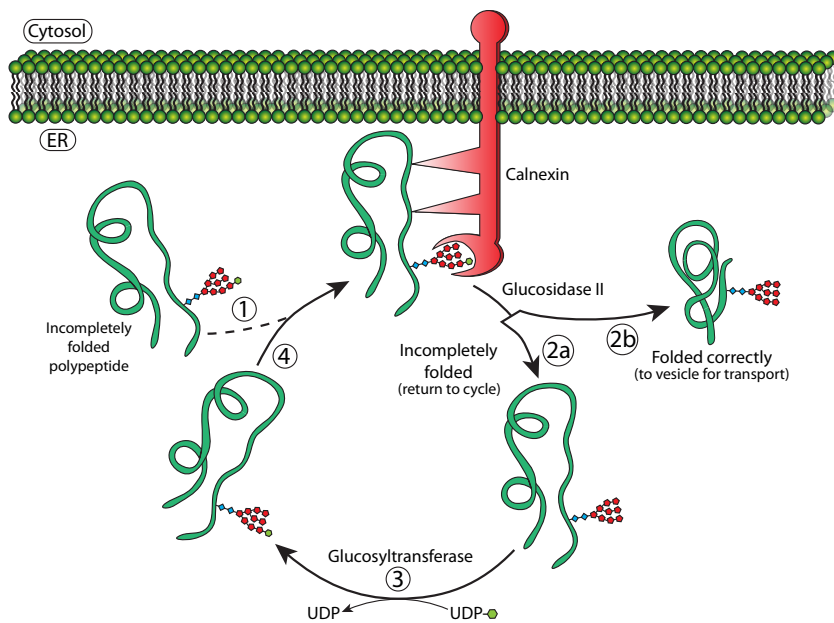

Figure 13. N-glycosylation can be used in error-checking.

Most glycoproteins continue with oligosaccharide remodeling once they have been moved from the ER to the Golgi apparatus by vesicular transport. There, a variety of glycosidases and glycosyltransferases prune and add to the oligosaccharide. Although the glycosylation is consistent and stereotyped for a given protein, it is still unclear exactly how the glycosylation patterns are determined.
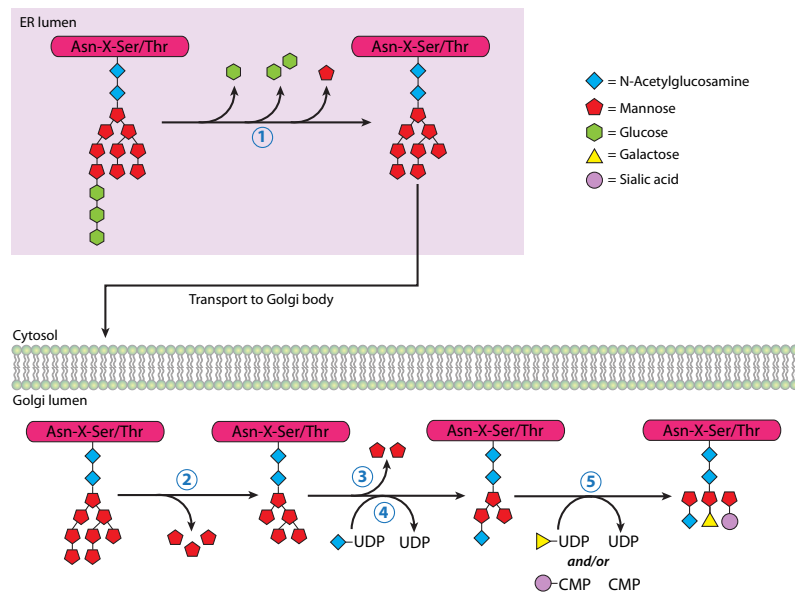

Figure 14. N-linked glycosylation can continue in the Golgi. Sugars may be added and removed in different patterns by glycosyltransferases resident in the Golgi.

O-linked Protein Glycosylation takes place entirely in the Golgi

O-linked glycoproteins begin their glycosylation with the action of the Golgi-specific enzyme, GalNAc transferase, which attaches an N-acetylgalactosamine to the hydroxyl group of a serine or threonine. The determination of which residue to glycosylate appears to be directed by secondary and tertiary structure as previously mentioned, and often occurs in dense clusters of glycosylation. Despite being fairly small additions (usu. <5 residues), the combined oligosaccharide chains attached to an O-linked glycoprotein can contribute over 50% of the mass of a glycoprotein. Two of the better known O-linked glycoproteins are mucin, a component of saliva, and ZP3, a component of the zona pellucida (which protects egg cells). These two examples also illustrate a key property of glycoproteins and glycolipids in general: the sugars are highly hydrophilic and hold water molecules to them, greatly expanding the volume of the protein.

Two common antibiotics, tunicamycin and bacitracin, can target N-linked glycosylation, although their antibiotic properties come from disrupting formation of bacterial cell walls. Tunicamycin is an analogue of UDP-GlcNAc, and inside eukaryotic cells can disrupt the initial oligosaccharide formation by blocking the initial GlcNAc addition to the dolichol-phosphate. Since it can be transported into eukaryotic cells, tunicamycin is not clinically useful due to its toxicity. Bacitracin, on the other hand, is a small cyclic polypeptide that binds to dolichol-PP preventing its dephosphorylation to dolichol-P, which is needed to build the oligosaccharide. Bacitracin is not cell-permeable, so even though it has similar activity to tunicamycin on bacteria by disrupting extracellular glycolipid synthesis needed for cell wall formation, it is harmless to eukaryotes and thus is a useful therapeutic antibiotic.
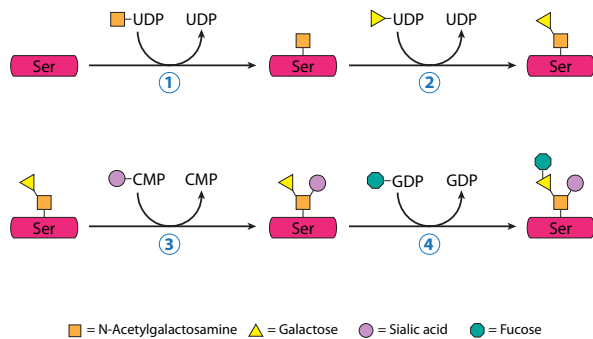
Figure 15. O-linked glycosylation in the Golgi involves attachment of only a few sugars to serine or threonine.

■ = N-Acetylgalactosamine △ = Galactose ○ = Sialic acid ● = Fucose

Interestingly, this protective waterlogged shell can mask parts of the protein core. In the case of the cell adhesion molecule, NCAM, which is a highly polysialylated glycoprotein at certain developmental stages and locations, and unglycosylated in others, the naked protein can be recognized as an adhesive substrate while the glycosylated protein can be recognized as a repulsive substrate to other cells. Even in highly glycosylated proteins though, the sugar residues often acts as recognition sites for other cells. For instance, the zona pellucida is very important as a physical barrier that protects the egg, but glycosylated ZP3 also acts as a sperm receptor.

Vesicular Transport

In addition to protein processing, the ER and Golgi also take care of some types of protein transport. Vesicles (membrane-bound bubbles, essentially) pinch off from the ER, Golgi, and other membranous organelles, carrying with them whatever soluble molecules were inside the fluid that was enclosed as well as any molecules embedded in that section of membrane. These vesicles then catch a ride on a molecular motor such as kinesin or myosin, and travel along the cytoskeleton until they dock at the appropriate destination and fuse with the target membrane or organelle. In general, vesicles move from the ER to the cis-Golgi, from the cis- to the medial Golgi, from the medial to the trans- Golgi, and from the trans-Golgi to the plasma membrane or other compartments. Although most movement is in this direction, there are also vesicles that move back from the Golgi to the ER, carrying proteins that were supposed to stay in the ER (e.g. PDI) and were accidentally scooped up within a vesicle.

The formation of vesicles is dependent on coat proteins that will, under proper conditions, self-assemble into spherical cages. When associated with transmembrane proteins, they can pull the attached membrane along into a spherical shape also. The major types of coat proteins used in vesicle formation are COPII, COPI, and clathrin.
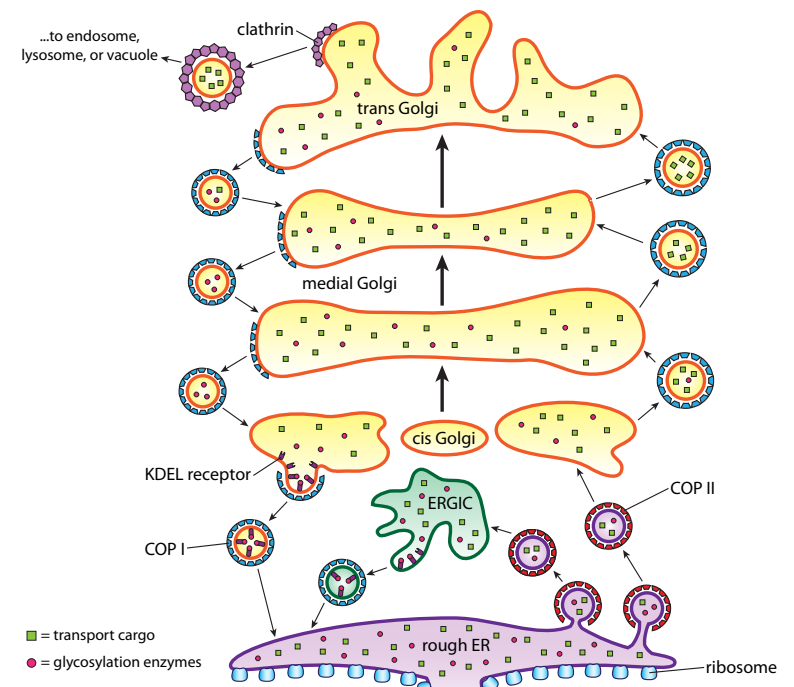

Figure 16. Vesicles bud from the endoplasmic reticulum and merge to form ERGIC, which matures into the cis Golgi, then the medial Golgi, and finally the trans Golgi. Vesicles may also bud from any of these other compartments to other organelles or to the plasma membrane.

■ = transport cargo
● = glycosylation enzymes

COPII coat proteins form the vesicles that move from ER to Golgi. COPI coat proteins are used between parts of the Golgi apparatus as well as to form vesicles going from the Golgi back to the ER. Finally, clathrin is used to form vesicles leaving the Golgi for the plasma membrane as well as for vesicles formed from the plasma membrane for endocytosis.
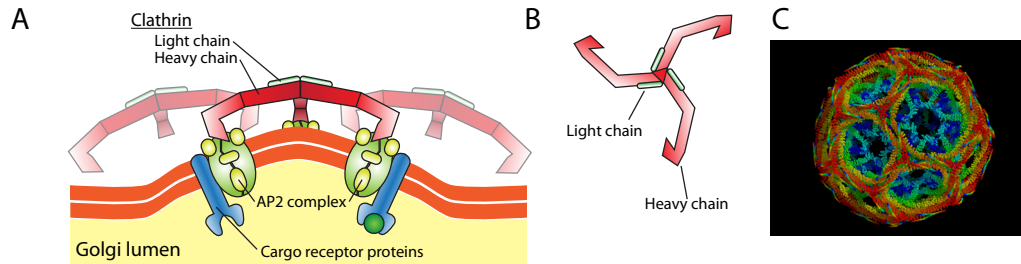

Figure 17. Clathrin. (A) clathrin binds to adapter proteins which are bound to transmembrane cargo receptors, linking the membrane with the clathrin. (B) A single clathrin triskelion is composed of three heavy chains and three light chains. (c) The triskelions self-assemble into a roughly spherical construct without the need for any additional energy or enzymes.

Clathrin (fig. 17) is the best described of the three, and the vesicular coats are made from arrangements of clathrin triskelions (from Gk. meaning three-legged). Each triskelion is composed of three heavy chains joined together at the C-terminus, and three light chains, one associated with each heavy chain. The heavy chains of different triskelions interact along the length of their heavy chain "legs" to create a very sturdy construct. The light chains are unnecessary for vesicle formation, and are thought to help prevent accidental interactions of clathrin molecules in the cytoplasm.

There is significant similarity between the vesicle formation mechanisms using these different coat proteins, beginning with the recruitment of ARF1 (ARF stands for ADP ribosylation factor, which has nothing to do with its function here) to the membrane. This requires the ARNO-facilitated exchange of a GTP for GDP (ARNO is ARF nucleotide binding site opener). Once ARF1 has bound GTP, the conformational change reveals an N-terminal myristoyl group which inserts into the membrane. Both COPI and clathrin-coated vesicles use ARF1 and ARNO, but COPII uses similar proteins called Sar1p and Sec12p.

The ARF1 (or Sar1p) is used to recruit adapter proteins that bind to the "tail" end of membrane-bound receptor proteins. The business end of these receptors binds to cargo molecules that need to be packaged into the vesicle. The adapter proteins act as the link between the membrane (through the receptors) and the coat proteins. For clathrin, the adapter proteins are AP1 for trans-Golgi-derived vesicles and AP2 for endocytic

From the late 1990's, there has been debate about the origins and stability of the Golgi apparatus. The two primary competing models, both of which are described in many textbooks prior to 2006, were the stable compartment model and the cisternal maturational model. According to the older stable compartment model, the cis, medial, and trans Golgi are each permanent structures with particular characteristics (lumenal enzymes, pH, etc). Proteins are shuttled from cis to medial to trans compartments in sequence. The cisternal maturation model, on the other hand, posits that cis Golgi compartments actually mature into medial Golgi, which then mature into trans Golgi - "maturation" being accomplished by import and export of specific proteins characteristic of a particular compartment. Although there was some evidence on either side of the argument, visual demonstration (fluorescent labeling of early- and late- Golgi specific proteins) of cisternal maturation in 2006 (Losev, et al, *Nature* **441**: 939-40) confirmed that model.
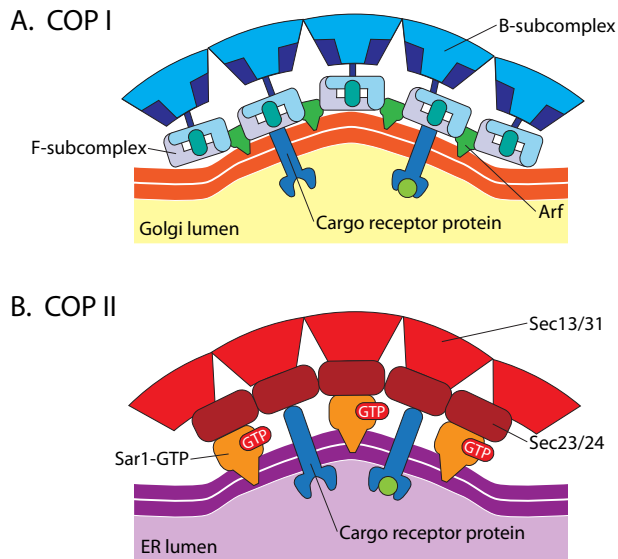
A. COP I

B-subcomplex

F-subcomplex

Golgi lumen

Cargo receptor protein

Arf

B. COP II

Sec13/31

GTP

Sar1-GTP

GTP

GTP

Sec23/24

Cargo receptor protein

ER lumen

Figure 18. COP coated vesicles.

= Glycerophospholipids   = Sphingolipids   = Other membrane lipids

Figure 20. Glycerophospholipids are made primarily in the ER. Although the ER also makes the ceramide precursors for sphingolipids, the sphingolipids are made only in the Golgi.

vesicles. For COPI vesicles, the approximate homologues are the β-, γ-, δ-, and ζ- COPs while the COPII system uses Sec23p and Sec24p.

Finally, the adapters link to the actual coat proteins: clathrin, α- or ε- COP, Sec13p and Sec31p. What these proteins all have in common is that spontaneously (i.e. without any requirement for energy expenditure), they self-assemble into cage-like spherical structures. Under the electron microscope, the clathrin-coated vesicles are more sharply defined and the hexagonal and pentagonal shapes bounded by the clathrin subunits give the vesicle a "soccer ball" look. COP coatamer-coated vesicles are much fuzzier in appearance under EM.

All three types of vesicle coat proteins have the abiliy to spontaneously associate into a spherical construct, but only the COPI and COPII coated vesicle also spontaneously "pinch off" the membrane to release the vesicle from its originating membrane. Clathrin-coated vesicles require an external mechanism to release the vesicle (fig. 19). Once the vesicle has almost completed, there is still a small stalk or neck of membrane that connects the vesicle to the membrane. Around this stalk, dynamin-GTP molecules aggregate in a ring/spiral construction. Dynamin molecules are globular GTPases that contract upon hydrolysis of GTP. When they associate around the vesicle stalk, each dynamin protein contracts, with the combined effect of constricting the stalk enough that the membrane pinches together, sealing off and releasing the vesicle from the originating membrane.
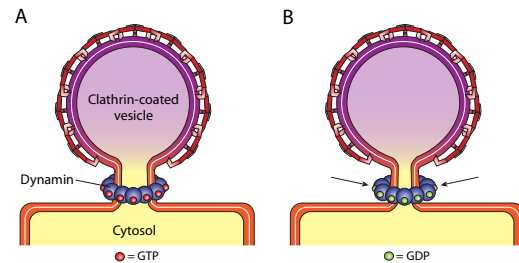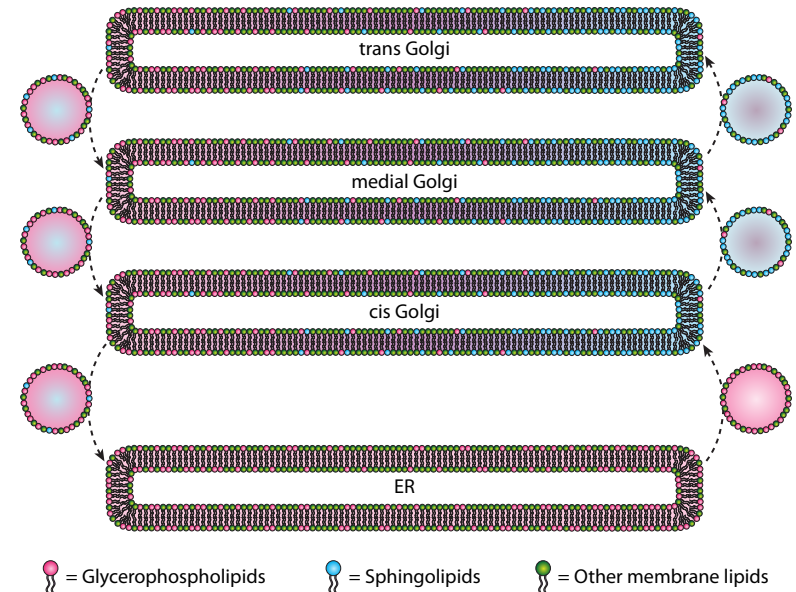

A

B

Clathrin-coated vesicle

Dynamin

Cytosol

= GTP

= GDP

Figure 19. Dynamin monomers, each of which is a GTPase, polymerizes around the neck of the vesicle. When the GTP is hydrolyzed, the dynamin "noose" tightens and pinches off the vesicle.

Although lipids and membranes were discussed in chapter 4, we neglected to discuss the location of their syntheses in eukaryotes. As fig. 20 indicates, the synthesis of certain types of lipids is segregated and exclusive. Glycerophospholipids are primarily formed in the endoplasmic reticulum, although they are also made in mitochondria and peroxisomes. In contrast, sphingolipids are not made in the ER (though their ceramide precursors are) in mammals, the necessary enzymes are found in the lumen of the cis and medial Golgi. There is evidence of anterograde and retrograde vesicular traffic between the various Golgi and ER compartments, which would theoretically indicate a redistribution of lipid types. However, the sphingolipids tend to aggregate into lipid rafts and seem to be more concentrated in anterograde-moving vesicles.

The coat proteins come off shortly after vesicular release. For clathrin, the process involves Hsc70, an ATPase. However, for COPI or COPII coated vesicles, hydrolysis of the GTP on ARF/Sar1p appears to weaken the coat protein affinity for the adapters and initiates uncoating. The GTPase activator is ARF GAP (or Sec23p) and is an integral part of the COP I (or II) coat.

The vesicles carry two categories of cargo: soluble proteins and transmembrane proteins. Of the soluble proteins, some are taken up in the vesicle by virtue of being bound to a receptor. Other proteins just happen to be in the vicinity and are scooped up as the vesicle forms. Occasionally, a protein is taken up that was not supposed to be; for example, PDI may be enclosed in a vesicle forming from the ER. It has little function in the Golgi, and is needed in the ER, so what happens to it? Fortunately, PDI and many other ER proteins have a C-terminal signal sequence, KDEL (Lysine-Aspartic Acid-Glutamic Acid-Leucine), that screams "I belong in the ER." This sequence is recognized by KDEL receptors inside the Golgi, and binding of the KDEL proteins to the receptors triggers vesicle formation to send them back to the ER.

Secretory vesicles have a special problem with soluble cargo. If the vesicle was to rely simply on enclosing proteins within it during the formation process, it would be difficult to get high concentrations of those proteins. Many secreted proteins are needed by the organism quickly and in significant amounts, so there is a mechanism in the trans Golgi for aggregating secretory proteins. The mechanism uses aggregating proteins such as secretogranin II and chromogranin B that bring together the target proteins in large concentrated granules. These granins work best in the trans Golgi milieu of low pH and high Ca++, so when the vesicle releases its contents outside of the cell, the higher pH and lower Ca++ breaks apart the aggregates to release the individual proteins.

There is a consistent pH change during the maturation of the Golgi, so that as we go from ER to Golgi, each compartment has a progressively lower (more acidic) lumenal pH.

Finally, there is the question of targeting the vesicles. The vesicles are much less useful if they are tossed on a molecular freight train and dropped off at random. Therefore, there is a docking mechanism that requires a matching of the v-SNARE protein on the vesicle's cytoplasmic surface and the t-SNARE on the cytoplasmic surface of the target membrane. Fusion of the vesicle to the membrane only proceeds if there is a match. Otherwise, the vesicle cannot fuse, and will attach to another molecular motor to head to another, hopefully correct, destination. This process is aided by tethering proteins which initially make contact with an incoming vesicle and draw it close enough to the target to test for SNARE protein interaction. Other proteins on the vesicle and target membranes then interact and if the SNAREs match, can help to "winch" the vesicle into the target membrane, whereupon the membranes fuse. An important rule of thumb to understanding vesicular fusion and also the directionality of membrane proteins and
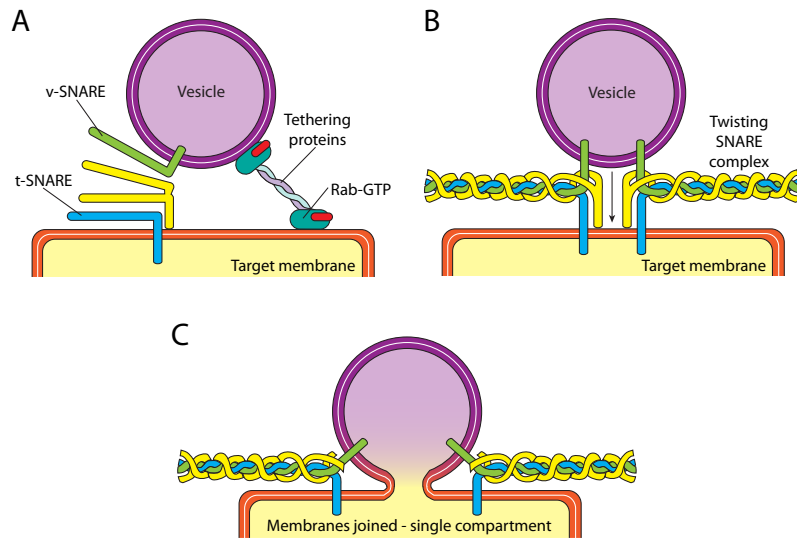
Figure 21. Vesicles first interact with tethering proteins (A), which help bring the vesicle and target membranes close. SNAREs can then interact, and if they match, then they will begin to twist around each other, ratcheting the two membranes closer as they twist.

lipids, is that the cytoplasmic-facing side of a membrane is always going to be facing the cytoplasm. Therefore a protein that is eventually found on the outer surface of the cell membrane will have been inserted into the lumenal surface of the ER membrane to begin with.

More specifically, as a vesicle approaches the target membrane, the tethering protein Rab-GTP, which is linked to the target membrane via a double geranylgeranyl lipid tail, loosely associates with the vesicle and holds it in the vicinity of the target membrane to give the SNARES a chance to work. The v-SNAREs and t-SNAREs now have the opportunity to interact and test for a match. Recently, the SNAREs have been renamed R-SNAREs and Q-SNAREs, respectively, based on conserved arginine and glutamine residues. In addition to these two primary SNAREs, at least one other SNARE is involved, together forming a bundle of four α-helices (four, not three, because at least in the best studied example, one of the SNAREs is bent around so that two of its alpha-helical domains participate in the interaction. The four helices wrap around each other and it is thought that as they do so, they pull the vesicle and the target membrane together.

The tetanus toxin, tetanospasmin, which is released by Clostridium tetani bacteria, causes spasms by acting on nerve cells, and preventing neurotransmitter release. The mechanism for this is that it cleaves synaptobrevin, a SNARE protein, so that the synaptic vesicles cannot fuse with the cell membrane. Botulinum toxin, from Clostridium botulinum, also acts on SNAREs to prevent vesicle fusion and neurotransmitter release, although it targets different neurons and so has the opposite effect: tetanus is caused by preventing the release of inhibitory neurotransmitters, while botulism is caused by preventing release of excitatory neurotransmitters.

Receptor-mediated Endocytosis

Just as there is vesicular traffic towards the plasma membrane, either for secretion or for incorporation of membrane lipids or proteins, there can also be vesicular traffic from the plasma membrane. Endocytosis is the process by which a coat protein (usually clathrin) on the cytoplasmic side of the plasma membrane, begins to polymerize a coat that draws the membrane with it into a vesicle. However, instead of capturing a bit of ER or Golgi lumen with it, the vesicle contains a little material from outside of the cell. Sometimes endocytosis is initiated internally, perhaps to remove a particular protein from the cell surface (for an example, see trailing edge dynamics in cell motility in the next chapter), but often, the endocytosis is the result of a ligand binding to an extracellular receptor molecule, leading to its activation and subsequent nucleation of a clathrin assembly and vesicle formation.

There are many types of ligands: a nutrient molecule (usually on a carrier protein, as in the examples below) or even an attacking virus which has co-opted the endocytic mechanism to facilitate entry into the cell. The example depicted here is a classic example: endocytosis of cholesterol (via low-density lipoprotein). This illustrates one potential pathway that the receptors and their cargo may take. In the case of cholesterol, the carrier protein is broken down fully, although in the case of transferrin, a serum protein that carries iron in the blood, the carrier protein is just recycled after releasing its transferrin cargo. It is packaged into an exocytic vesicle headed back to the cell surface.

Serum cholesterol is usually esterified and bound by LDL (low density lipoprotein), which then floats about in the bloodstream until it meets up with an LDL receptor on the surface of a cell. When the LDL binds to its receptor, the receptor is activated, and a clathrin-coated vesicle forms around the LDL/receptor complex. LDL receptors tend to aggregate in what are known as clathrin-coated pits — crater-like partial vesicles that already have a small number of polymerized clathrin molecules. The vesicle forms exactly as described previously for Golgi-derived clathrin vesicles: the clathrin self-assembles into a spherical vesicle, and dynamin pinches the vesicle off the cell membrane. This vesicle then fuses with an early endosome, which carries proton pumps in its membrane, causing the environment inside the vesicle to acidify (~pH 6). This acidification can cause conformational shifts in proteins that could, for example, lead to a receptor releasing its ligand, as is the case here with LDL and LDL receptor. The early endosome also functions as a sorting station: the receptor is re-vesicularized and transported back to the plasma membrane. Meanwhile, the LDL is packaged into a different vesicle and heads off for further processing.

The endosomal proton pumps are ATP-driven, Mg^{++}-dependent V-type pump (as opposed to the F-type pump in the mitochondrial inner membrane). Structurally, the two are similar though, and ATP hydrolysis drives the rotary unit, which then powers the movement of protons across the membrane from cytoplasm into endosome.
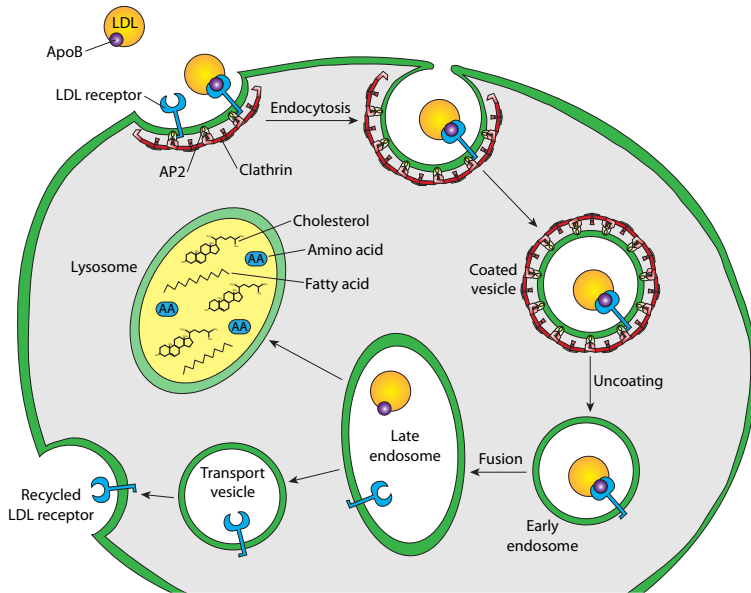
Figure 22. Receptor-mediated endocytosis of cholesterol via low-density lipoprotein.

The endosomal vesicle with the LDL in it next fuses with another acidic, membrane-bound compartment. The lysosome, at pH ~5.0, is even more acidic than the endosome, and it also contains a large complement of acid hydrolases - hydrolytic enzymes ranging across substrates (incl. proteases, lipases, glycosidases, nucleases) that operate optimally in acidic conditions, and minimally in the neutral or slightly basic conditions in the cytoplasm. In part, this is a safety mechanism — leakage of digestive enzymes from the lysosome will not result in wholesale digestion of the cell because the enzymes have little or no activity in the cytoplasm. The lysosomal membrane, in addition to having proton pumps to acidify the internal environment, also incorporates many transporter proteins to aid in moving the digestion products of the acid hydrolases out of the lysosome so that the cell can make use of the amino acids, sugars, nucleotides, and lipids that result. Back to our example, that means that the cholesterol esters are broken apart into individual cholesterol molecules, and the lipoprotein is broken down into lipids and amino acids. Interestingly, these transporter proteins are not digested by the lysosomal proteases because they are very heavily glycosylated, which shields potential proteolytic sites from the proteases.

Since it depends greatly on the contents of the endosome(s) that fused with it, the size and contents of lysosomes can vary greatly. In fact, the lysosome may also degrade internal cellular components through the process of *autophagy*. Usually, this is initi-

Lysosomal enzymes are specifically tagged by a mannose-6-phosphate that is added in the cis Golgi. This is a two-step process in which N-acetylglucosamine phosphotransferase adds a phospho-GlcNAc to a mannose residue, connecting via the phosphate group, then a phosphodiesterase removes the GlcNAc, leaving the mannose-6-P. This specifically targets lysosomal enzymes because they all have specific protein recognition sequences that the phosphotransferase binds to before transferring the P-GlcNAc. Although the lysosomal enzymes are tagged in the cis Golgi, they do not sort until the trans Golgi, when mannose-6-P receptors bind to the lysosomal enzymes and form lysosomal vesicles that will bud off and travel to late endosomes and lysosomes to deliver their acid hydrolase payload. Again, the pH change is important: in the somewhat acidic (pH 6.5) environment of the trans Golgi, the receptor binds the mannose-6-P-tagged enzymes, but in the more acidic lysosome, the acid hydrolases are released to do their work.

When one or more acid hydrolases do not function properly or do not make it into the lysosome sue to improper sorting, the result is incomplete digestion of the lysosomal contents. This in turn leads to the formation of large inclusions of partially digested material inside the lysosomes. This accumulation of material can be cytotoxic, and genetic disorders that affect the expression or sorting of lysosomal hydrolases are collectively referred to as lysosomal storage diseases. These fall into several categories depending on the types of molecules accumulated.

A common and easily treatable disease of glycosaminoglycan accumulation is Hurler's disease, which can be effectively treated and non-neurological effects even reversed by enzyme replacement therapy. Hurler's others in its class affect a wide variety of tissues because glycosaminoglycans are ubiquitous. On the other hand, because the brain is enriched in gangliosides, lysosomal storage diseases like Gaucher's disease show defects primarily in the CNS. Many lysosomal storage diseases have similar presentation: developmental abnormalities, esp. stunted bone growth, lack of fine facial features, and neuromuscular weakness.

ated under starvation conditions which lead to inhibition of mTor, and subsequent expression of autophagic genes. These then interact with mitochondria and other cellular components, and promote the formation of a double-membraned autophagosome around them. The origin of the membranes is unclear, although the ER is suspected. Finally, the autophagosome fuses with a lysosome, and the acid hydrolases break down the cell parts for energy. A variation on this called microautophagy can also occur, in which the lysosome itself invaginates a bit of cytoplasmic material and internalizes an intralysosomal vesicle that is then broken down.

Finally, it should be noted that the large vacuoles of plant cells are in fact specialized lysosomes. Recall that vacuoles help to maintain the turgor, or outward water pressure on the cell walls that lead to a rigid plant part rather than a limp, wilted one. One of the ways in which this occurs is that the acid hydrolases inside the vacuole alter the osmotic pressure inside the vacuole to regulate the movement of water either in or out.

Another example of receptor-mediated endocytosis is the import of iron into a mammalian cell. As with serum cholesterol, iron is not generally imported into the cell by itself. Instead, it is bound to apotransferrin, a serum protein that binds two Fe^{3+} ions. Once it has bound the iron ions, the apotransferrin is now referred to as transferrin, and it can be recognized and bound by transferrin receptors (TfR) located on the extracellular surface of cell membranes. This initiates receptor-mediated endocytosis just as described above. However, in this case, the lysosome is not involved. As the transferrin and transferrin receptor reach the early endosome, they do not dissociate, but rather the Fe^{2+} releases from the transferrin, and then exits the endosome via DMT1, a divalent metal transport protein to be used in heme groups or other complexes. This leaves the apotransferrin-TfR complex, which is recycled back to the cell membrane via vesicle. Once the vesicle fuses with the extracellular space, the acidity of the endosome is dissipated and the apotransferrin no longer binds to TfR. Apotransferrin can thus go back to its duty of finding iron ions and bringing them back to the cell.

The most severe, I-cell disease (mucolipidosis type II) occurs when nearly all lysosomal enzymes are missing in the fibroblasts of the affected individual. There is severe developmental delay and early growth failure, neuromuscular problems, and malformations in early skeletal development. The severity of this disorder is due to the almost complete lack of lysosomal enzymes, which is caused by a deficiency of GlcNAc phosphotransferase. Without it, no enzymes are tagged for sorting to the lysosome.

Other relatively common disorders include Tay-Sachs and Niemann-Pick diseases. Tay-Sachs is caused by an accumulation of gangliosides in the brain and is usually fatal by 5 years of age. Niemann-Pick, on the other hand, may manifest as Type A with an even shorter life expectancy, or as Type B, in which symptoms are relatively minor. The major difference is that Type A patients have very little (<5%) of their sphingomyelinase activity, while Type B patients have only slightly less than normal (~90%) activity.