

Glycosylation and Protein Folding

I. Introduction. As a translocated polypeptide emerges into the lumen of the ER, it is generally processed in three ways: 1) its signal sequence is cleaved by signal peptidase; 2) it is glycosylated; and 3) it must be helped to fold into the correct conformation.

II. Signal peptidase. Cleavage of the signal peptide is carried out by the membrane enzyme, signal peptidase, that is associated with the Sec61 complex with its active site in the lumen of the ER. This cleavage occurs co-translationally because even damaged proteins that never emerge from the Sec61 channel can have had their signal sequences cleaved off. The recognition sequence for cleavage consists of the two small amino acids at positions -1 and -3.

III. Glycosylation. Many secreted and membrane proteins have attached carbohydrate and are thus glycoproteins. There are two main types of sugar linkages: O-linked and N-linked.

A. O-linked The -OH of the amino acids serine or threonine can be connected to the C1 carbon of a sugar residue through a glycosidic bond. These types of sugar additions are primarily formed in the Golgi apparatus, which we will discuss later.

B. N-linked The amino acid asparagine can be linked to sugar through a glycosidic bond to the amide group of its side chain. (See **MBOC p. 589, Fig 12-48.**) This type of sugar addition is carried out by the membrane protein oligosaccharyl transferase which is associated with the Sec61 complex, with its active site in the lumen of the ER. Not all Asn residues become glycosylated. In order to be a substrate for glycosylation, the Asn must be part of the sequence: - Asn - X - Ser/Thr - . Not even every Asn in such a sequence is actually glycosylated although predicting which will or won't be is not currently precise.

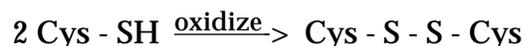
A very specific 14-sugar oligosaccharide is transferred to the Asn residue. (See **MBOC p. 589, Fig 12-48.**) It is composed of two N-acetylglucosamines (GlcNAc), followed by a branched network of 9 mannoses, and attached to one mannose are three glucoses. This oligosaccharide is transferred to the polypeptide from a molecule of dolichol (see **MBOC p. 590, Fig 12-49**). Dolichol is a hydrophobic compound made of 18 - 20 repeating isoprenoid units (see **V&V p. 692 Fig. 23-28**) and resides in the ER membrane. The 14-sugar oligosaccharide is built onto a molecule of dolichol-phosphate. Sugar residues are activated by being complexed to nucleotide diphosphates and are then transferred from the nucleotide to the growing oligosaccharide. (See **MBOC p. 590, Fig 12-50**). The addition of sugars to dolichol begins on the cytoplasmic face of the ER membrane and at some point the dolichol with its attached sugars must "flip" through the membrane to the luminal side (**V&V p. 611 Fig. 21-15**). The protein machinery that must mediate this flipping has not been identified.

Once the oligosaccharide has been transferred to the protein, it is trimmed back. The loss of the three glucose molecules and one of the mannoses seems to be a signal required to move the protein out of the ER.

Tunicamycin is a fungal toxin that blocks the transfer of the first molecule of phosphorylated GlcNAc to dolichol, (see **V&V p. 614, Fig. 21-19**), which completely blocks the glycosylation of Asn residues. From studies where N-glycosylation was blocked with tunicamycin, it was found that proteins that are normally glycosylated were still functional without the attached sugar. So, why does a cell go to so much trouble to N-glycosylate proteins? Well, the sugar may make the proteins more resistant to proteases, but it certainly does help the protein make its way to the exterior of the cell. In the presence of tunicamycin, proteins were processed through the ER and Golgi less efficiently than normal.

IV. Protein Folding. At the beginning of the semester, you were told that all of the information required for protein folding is contained in a protein's primary sequence. Well, it is not actually that simple for all proteins, and proteins translocated into the ER find themselves in an oxidizing environment with a very high protein concentration. How does the cell try to ensure that they all fold correctly?

A. Glutathione The cytoplasm is a reducing environment, but the outside of a cell and, to a lesser extent, the interior of the ER and Golgi are oxidizing environments. A protein can use this oxidizing environment to help stabilize its structure by cross-linking its Cys residues via disulfide bridges in the following manner:

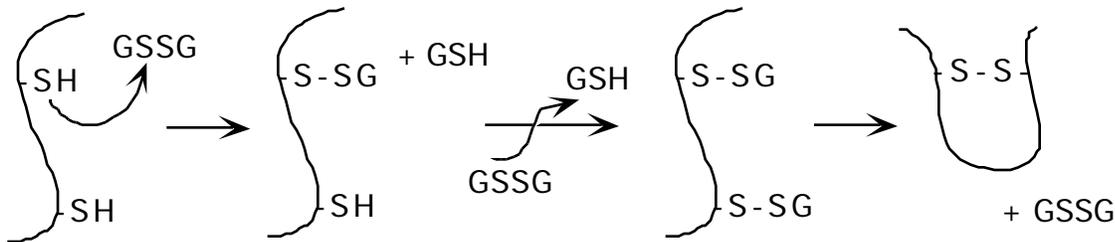


If the ER is changed to have a reducing environment, so that disulfide bonds cannot form, then proteins don't fold normally. If a protein only has two Cys residues then there is only one way to join them. However, if a protein has 4 Cys, then there is less than a 10% chance that the correct disulfide pattern will happen spontaneously. In order to form the correctly-folded protein, disulfide bond swapping must occur. This swapping is mediated by the small tripeptide glutathione, which has the sequence Glu - Cys - Gly. The reduced form of glutathione is abbreviated GSH while the oxidized form is abbreviated GSSG.

Thus,



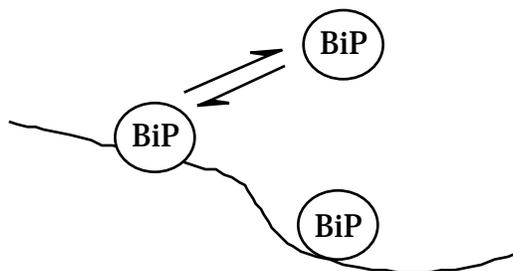
In the reducing cytoplasm, the ratio of GSH to GSSG is 100 to 1 (and the ratio of NADH to NAD⁺ is high as well); in the lumen of the ER, it is 1 to 1. So, Cys residues in the ER can interchange disulfide bonds through GSSG. For instance:



The exchange reaction is catalyzed by a protein called protein disulfide isomerase. This protein has its own -SH groups and can catalyze the rearrangement of a substrate protein's disulfides until that substrate protein achieves its most stable, native conformation.

B. Chaperones Chaperones are proteins that belong to the heat shock family and they catalyze the process by which newly formed proteins try out different folding arrangements until they ultimately fold into their correct structure. **BiP** (for binding protein) is the major protein-binding chaperone in the ER. The chaperone **calnexin**, also in the ER, binds to carbohydrates. The features of chaperones include:

- 1) **binding of peptides** - chaperones show saturable binding in the micromolar range to small peptides containing some hydrophobic residues.
- 2) **peptide-dependent ATPase activity** - if ATP is added to the system, the chaperone becomes an ATPase in the presence of peptide. The turnover rate is slow, about 0.2/minute for BiP, because the chaperone is basically a way to buy time for the protein to fold.
- 3) **peptide binding is inhibited by ATP** - the ADP-bound chaperone binds to the polypeptide, then it exchanges the ADP for ATP. The ATP-bound chaperone releases the peptide. ATP hydrolysis must occur before the chaperone can bind to the peptide again. So, the chaperone is coming on and off of the peptide. If the peptide folds incorrectly, then there will still be exposed regions of the peptide to which the chaperone can bind once again.



C. Peptidyl prolyl cis-trans isomerase (PPI) As we have learned before, prolines are not strictly amino acids, and the cyclic structure of the N- and C- groups render the cis- and trans- peptidyl-prolyl bond essentially energetically favourable. Nevertheless, some proteins are more stable in one conformation over the other, and PPIs help catalyze the conversion between the cis and trans configurations.

GroEL and GroES

Chaperones found in the cytoplasm work the same way as those in the ER. In *E. coli*, GroEL and GroES compose a large complex cytoplasmic chaperone that works as follows (see [MBOC p. 215, Fig 5-29 and 5-30.](#)): the unfolded protein goes into a hole in the middle of GroEL, and a cap of GroES locks it inside. The ATP bound to GroES takes about 15 seconds to hydrolyze. During this time, the protein is trapped inside trying to find its correct conformation. When the ATP is hydrolyzed, the GroES cap falls off. If the protein is folded incorrectly another GroEL takes it in and starts the process over. If after several tries the protein never folds correctly, proteases eventually recognize it as a mistake and chop it up.