11.4: 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 (Figure \(\PageIndex{12}\)) without being triggered by translation or protein entry.
Figure \(\PageIndex{12}\). 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.

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.

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 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.

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 (Figure \(\PageIndex{13}\)). 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 (Figure13, 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 protein disulfide isomerase-like activity comes from ERp57, which is technically a thiol oxidoreductase, but is functionally similar to PDI.
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.

![Diagram](https://bio.libretexts.org/Bookshelves/Cell_and_Molecular_Biology/Book%3A_Cells_-_Molecules_and_Mechanisms_(Wong)/1...)

Figure \((\PageIndex{13})\). 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.

![Diagram](https://bio.libretexts.org/Bookshelves/Cell_and_Molecular_Biology/Book%3A_Cells_-_Molecules_and_Mechanisms_(Wong)/1...)

Figure \((\PageIndex{14})\). N-linked glycosylation can continue in the Golgi. Sugars may be added and removed in different patterns by glycosyltransferases resident in the Golgi.

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.