CHEM 537
Carbohydrate Biochemistry and Glycobiology
Part III: Glycobiology, Glycoproteins & Glycoconjugates

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Slide Set 3b

Chapters 11 & 23: *Biochemistry*, Voet/Voet, 4th edition, 2011
Introduction to Glycobiology, Taylor/Drickhamer, 3rd edition, 2011

Mucins are large, heavily *O*-glycosylated proteins.

The primary purpose of many mucins is to retain water at surfaces that are exposed to the environment but are not sealed by moisture-impermeable layers (e.g., digestive tract, genital tract, respiratory system). They serve as lubricants and protect from invasion by microorganisms.

The polypeptide component: up to 10,000 aa; membrane-bound or secreted; contain tandem repeats of simple aa sequences rich in Ser and Thr; tandem repeats differ in sequence between mucin types; *O*- and *N*-glycosylation can occur outside the region of tandem repeats.

Biosynthetic machinery for protein *O*-glycosylation

Comparisons to protein *N*-glycosylation

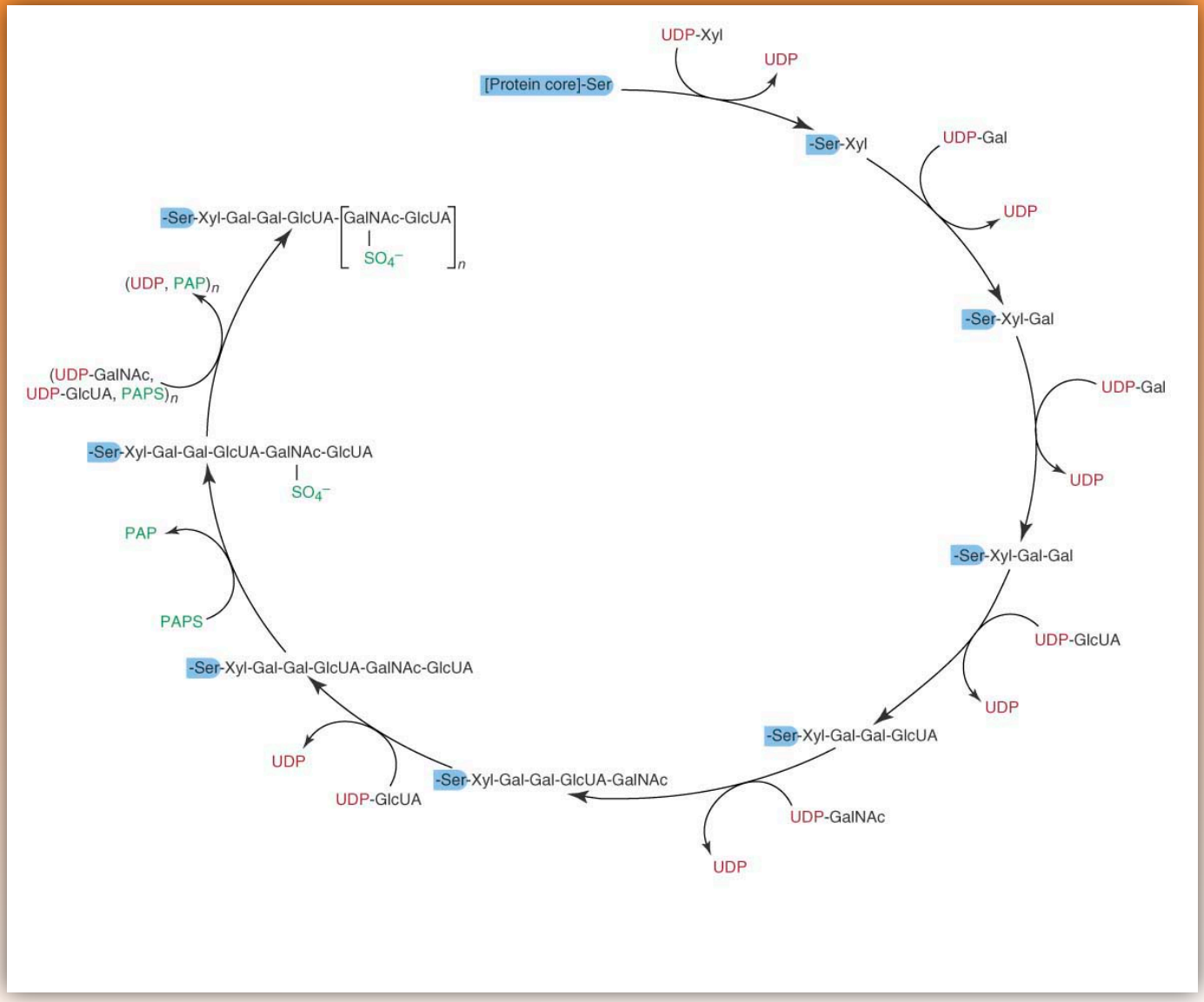
- Protein *O*-glycosylation involves glycosyltransferases analogous to those involved in protein *N*-glycosylation.

- Saccharide residues are added one at a time, starting from the initial GalNAc attached to Ser or Thr (there is no preformed core or *en bloc* transfer). There are numerous GalNAc transferases that attach the initial GalNAc to protein, each apparently displaying a unique specificity.

- There are no simple consensus sequences for *O*-glycosylation.

- *O*-Glycosylation occurs post-translationally in the Golgi.

Biosynthetic pathway for the synthesis of chondroitin sulfate proteoglycan



Biosynthetic route for the construction of a protein-bound chrondroitin sulfate oligosaccharide chain, showing sequential multiple additions of monosaccharide units

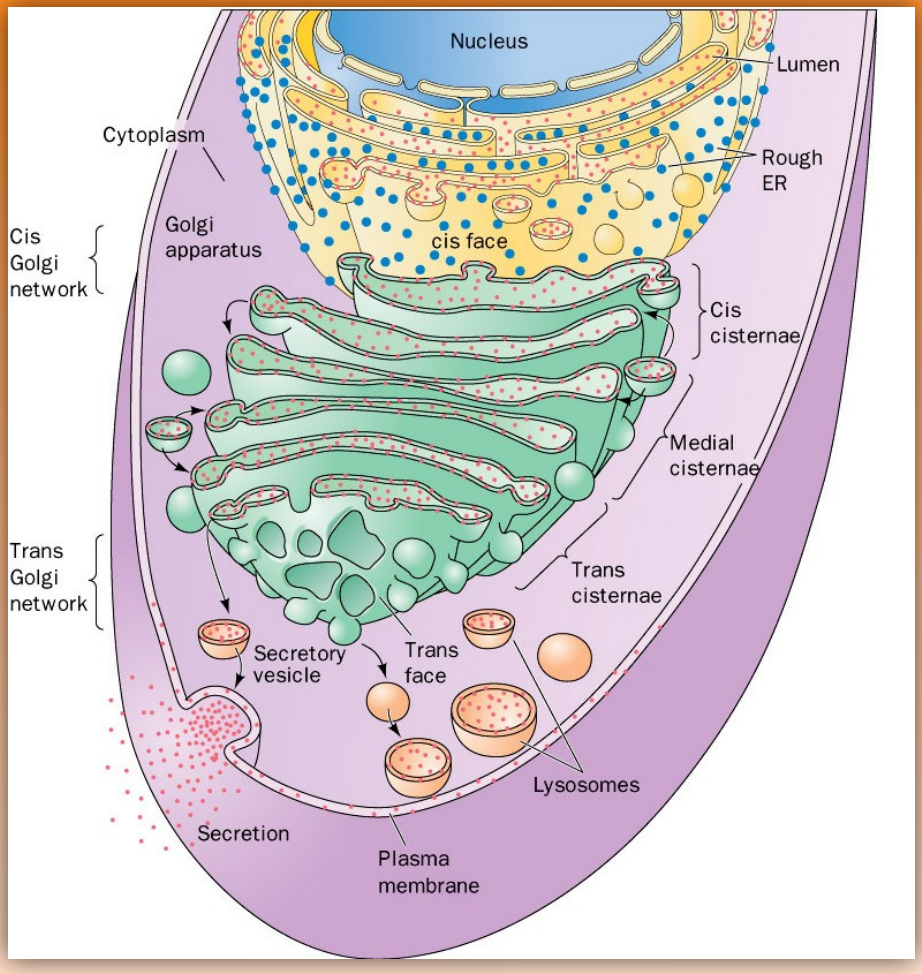
Biosynthesis of *N*-linked glycoproteins: Three stages

1. Formation of a lipid-linked precursor (parent) oligosaccharide ($Glc_3Man_9GlcNAc_2$)
2. *En bloc* transfer of the parent oligosaccharide to the polypeptide
3. Processing of the parent oligosaccharide; involves removal of some of the original saccharide residues (trimming by exoglycosidases) followed by addition of new saccharides (by glycosyltransferases) to the non-reducing termini of the glycan
4. The overall process occurs intracellularly in spacially differentiated steps.

The spacially-differentiated steps in *N*-linked glycoprotein biosynthesis

- **Rough ER**: lipid-linked precursor biosynthesis; *en bloc* transfer to protein; initial trimming reactions
- **Golgi apparatus** (*cis*, medial, *trans*): subsequent processing steps

Proteins destined for secretion, insertion into plasma membrane, or transport to lysosomes



Posttranslational processing of proteins

Synthesized by RER-associated ribosomes

During synthesis, proteins are either injected into the lumen or inserted into its membrane.

After initial processing in the ER, proteins are encapsulated into vesicles that bud from the ER and fuse with the *cis* Golgi network.

Progressive processing occurs in the *cis*, medial and *trans* cisternae of the Golgi.

In the *trans* Golgi, mature glycoproteins are sorted for delivery to plasma membrane, secretory vesicles or lysosomes; transported by other vesicles.

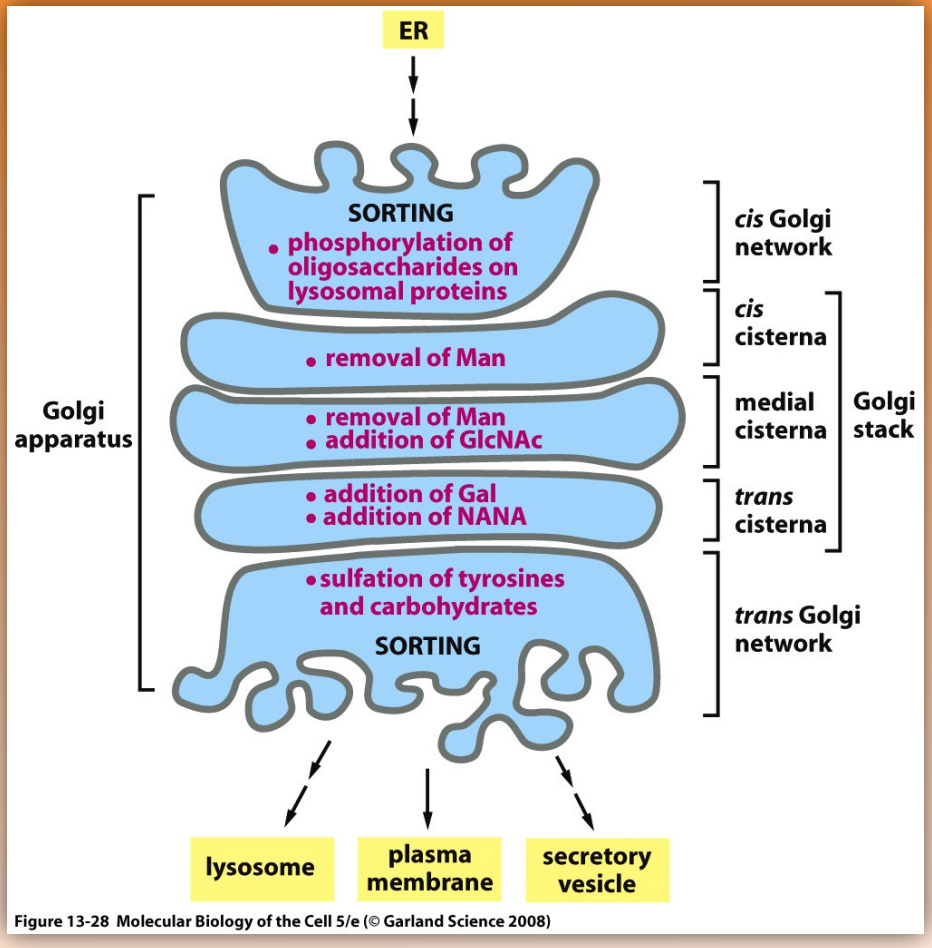
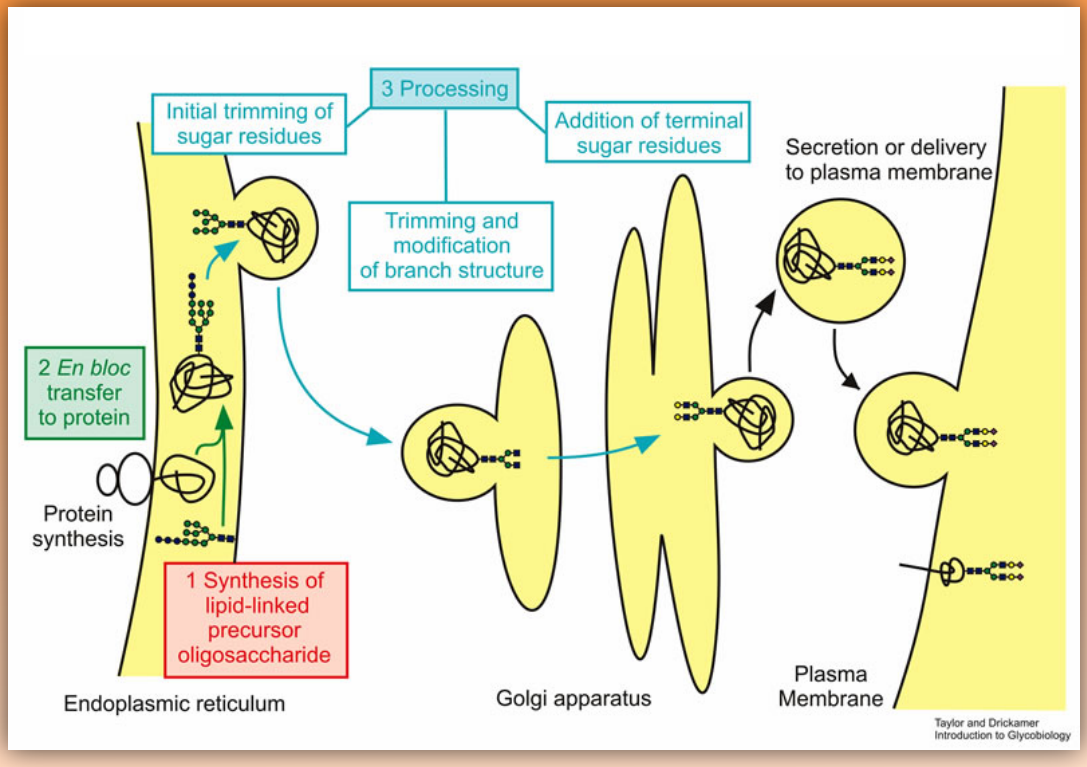


Figure 13-28 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Oligosaccharide processing in Golgi compartments

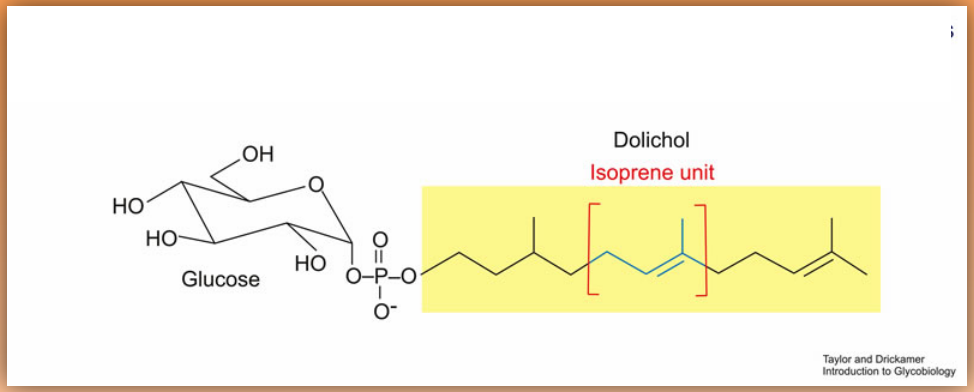
Processing enzymes are not spacially restricted to a particular cisternae; instead, their distribution is graded across the stack, such that early-acting enzymes are present mostly in the *cis* Golgi cisternae and later-acting enzymes are present mostly in the *trans* Golgi cisternae.

Initial attachment of an *N*-glycan to a protein is a co-translational event that occurs in the ER.

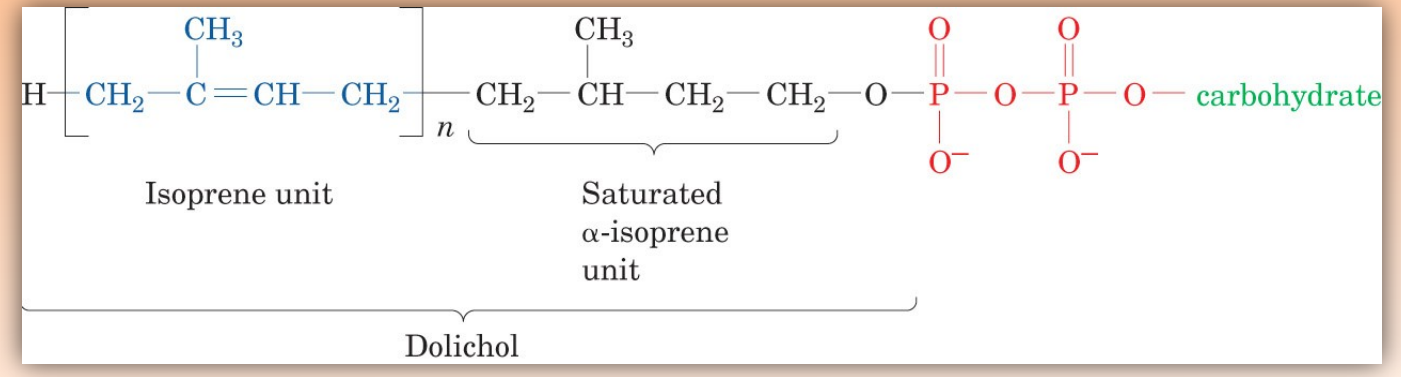


An overview of the pathway for glycoprotein biosynthesis and its intracellular location. Early stages involve glycan assembly on a glycolipid and subsequent transfer to nascent protein in the ER. Subsequent processing by glycosidases and glycosyltransferases occurs in the ER and Golgi apparatus.

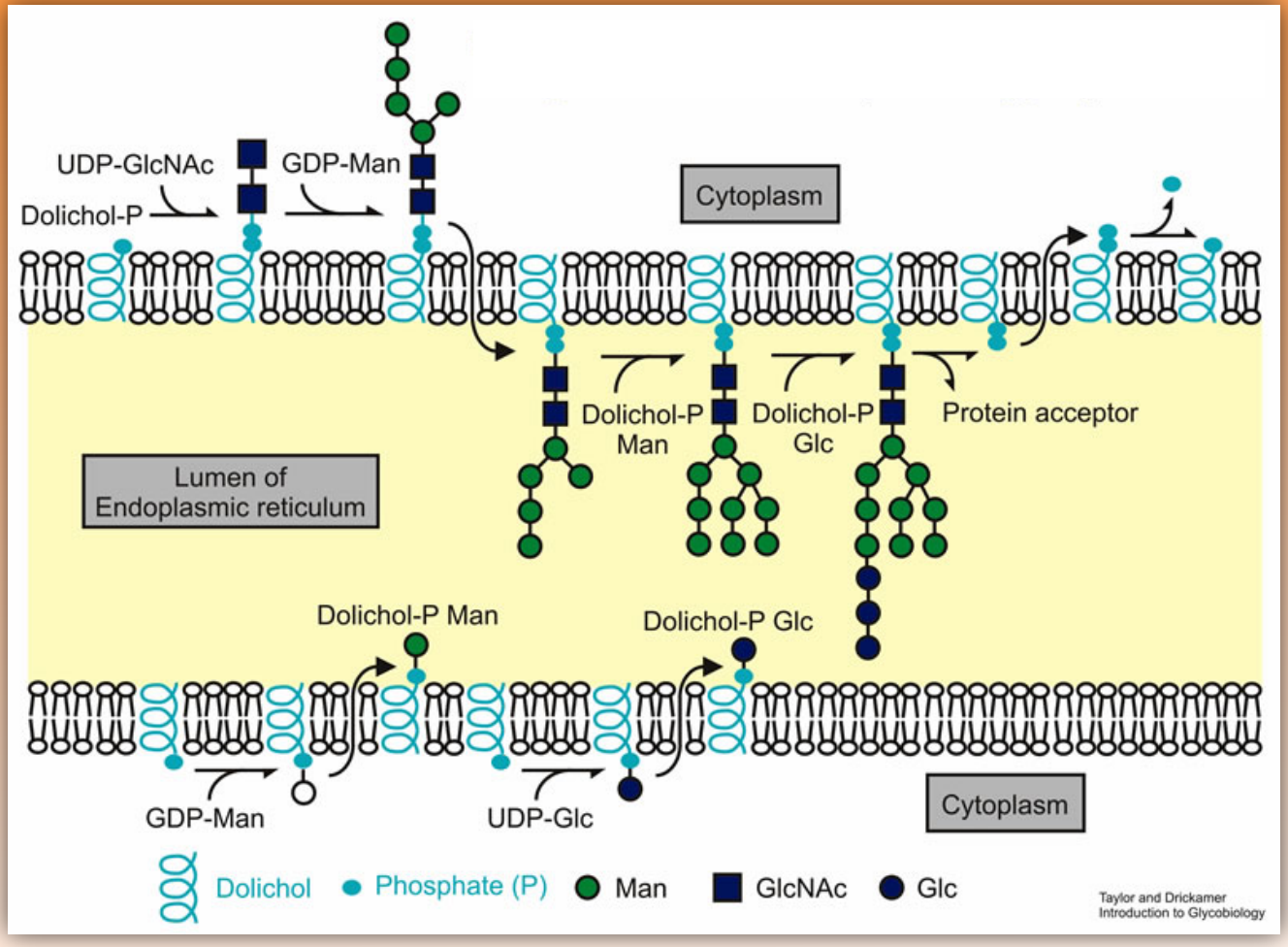
Dolichol derivatives serve as donors and carriers in the co-translational attachment of the parent *N*-glycan to nascent polypeptide on the luminal side of the ER membrane. Two kinds of glycosylated dolichols are involved: dolichol monophosphosugars and dolichol bisphosphosugars.



The long poly-isoprene tail, although far longer than the fatty acid tails of membrane phospholipids, is capable of lipid bilayer insertion, possibly in a helical or folded conformation.

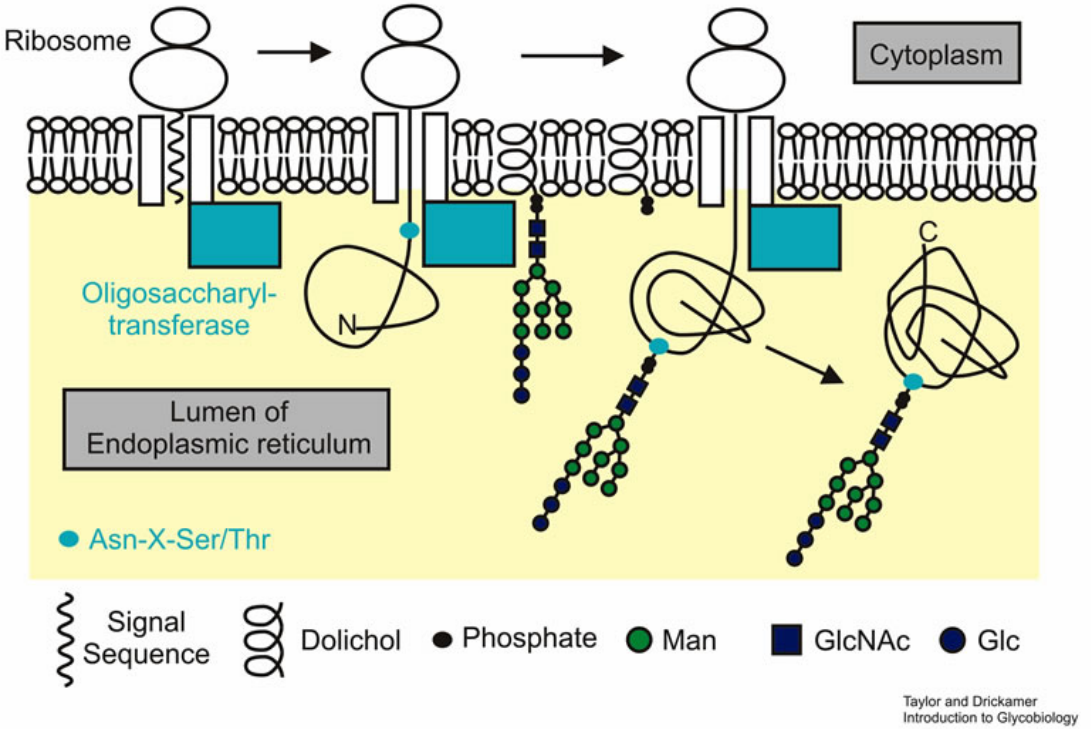


Generation of the dolichol-linked oligosaccharide donor (14-mer) for protein *N*-glycosylation: ER reactions



En bloc transfer of the precursor oligosaccharide (14-mer:$GlcNAc_2Man_9Glc_3$) is catalyzed by oligosaccharyl transferase (OST).

Consensus sequence: Asn-Xaa-Ser or Asn-Xaa-Thr, where Xaa can be any amino acid except Pro or Asp



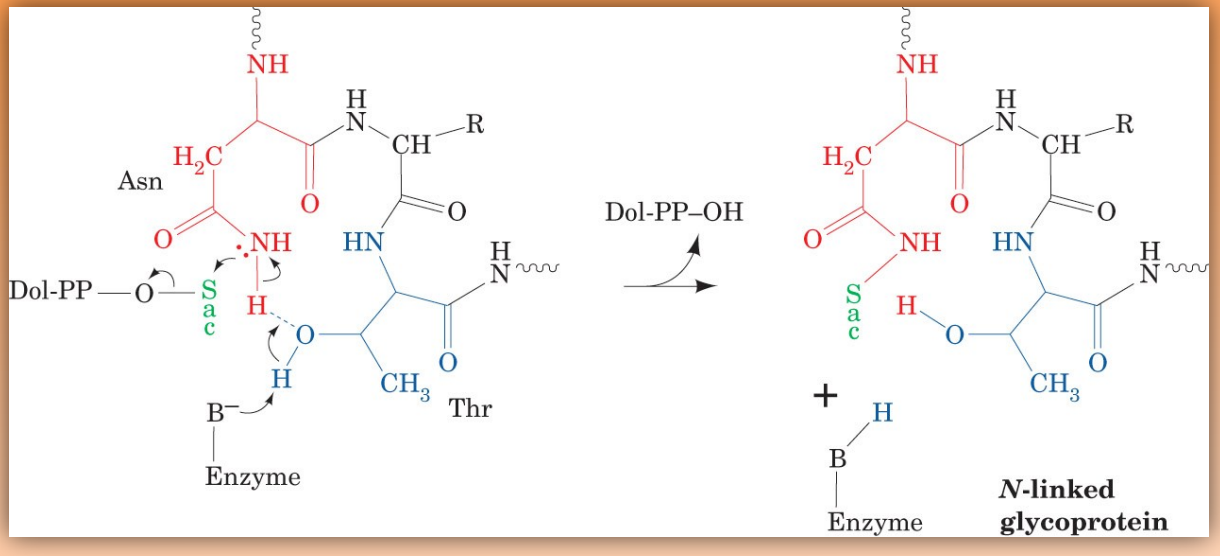
Co-translational addition of *N*-linked glycan to a nascent polypeptide

OST is associated with the channel through which the polypeptide is translocated to the ER lumen, so glycosylation occurs while the polypeptide is still unfolded.

N-Linked glycans are found at the surfaces of glycoproteins (not buried). Since transfer is co-translational involving presumably unfolded or partially folded protein, the mechanism for discrimination between consensus sites is unclear (*i.e.*, some consensus sequences are buried and unglycosylated).

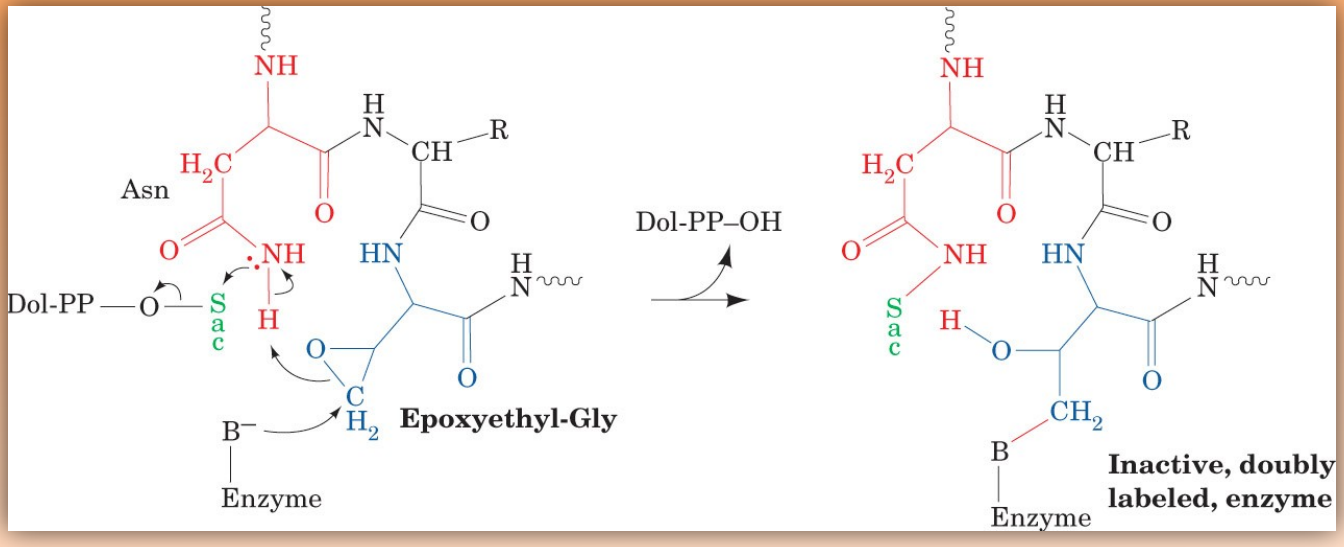
Mechanism of the oligosaccharyl transferase (OST) reaction

Chemical rationale for the Asn-X-Ser/Thr consensus sequence

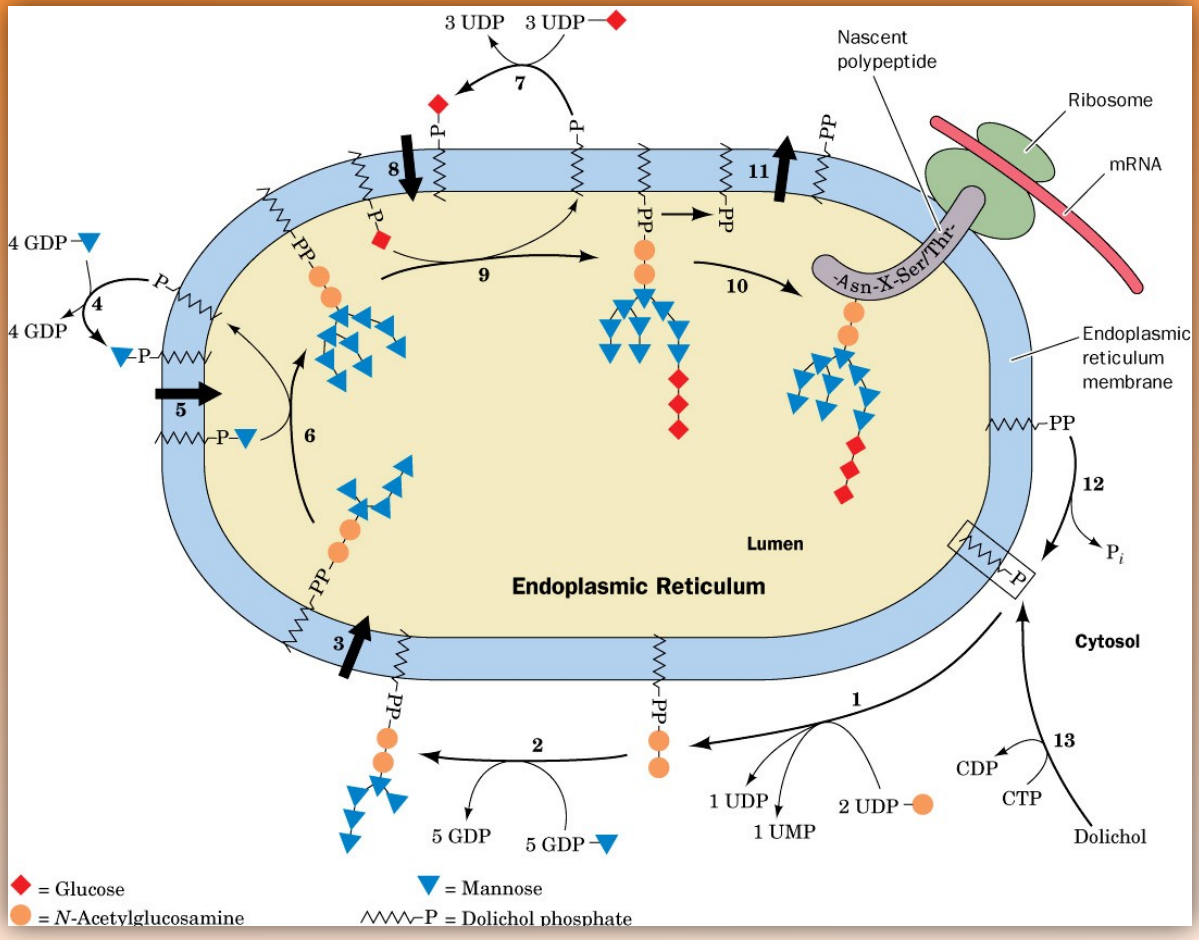


The Asn-X-Thr component of a hexapeptide model substrate forms a ring that is closed by an H-bond between the Asn side-chain amide hydrogens and the Thr hydroxyl group. A basic residue in the OST active site facilitates nucleophilic displacement of dolichol-PP from the oligosaccharide (Sac) by the Asn amide nitrogen, forming the *N*-glycosidic linkage.

Irreversible inactivation of OST by a hexapeptide containing Asn-Gly-epoxyethylGly

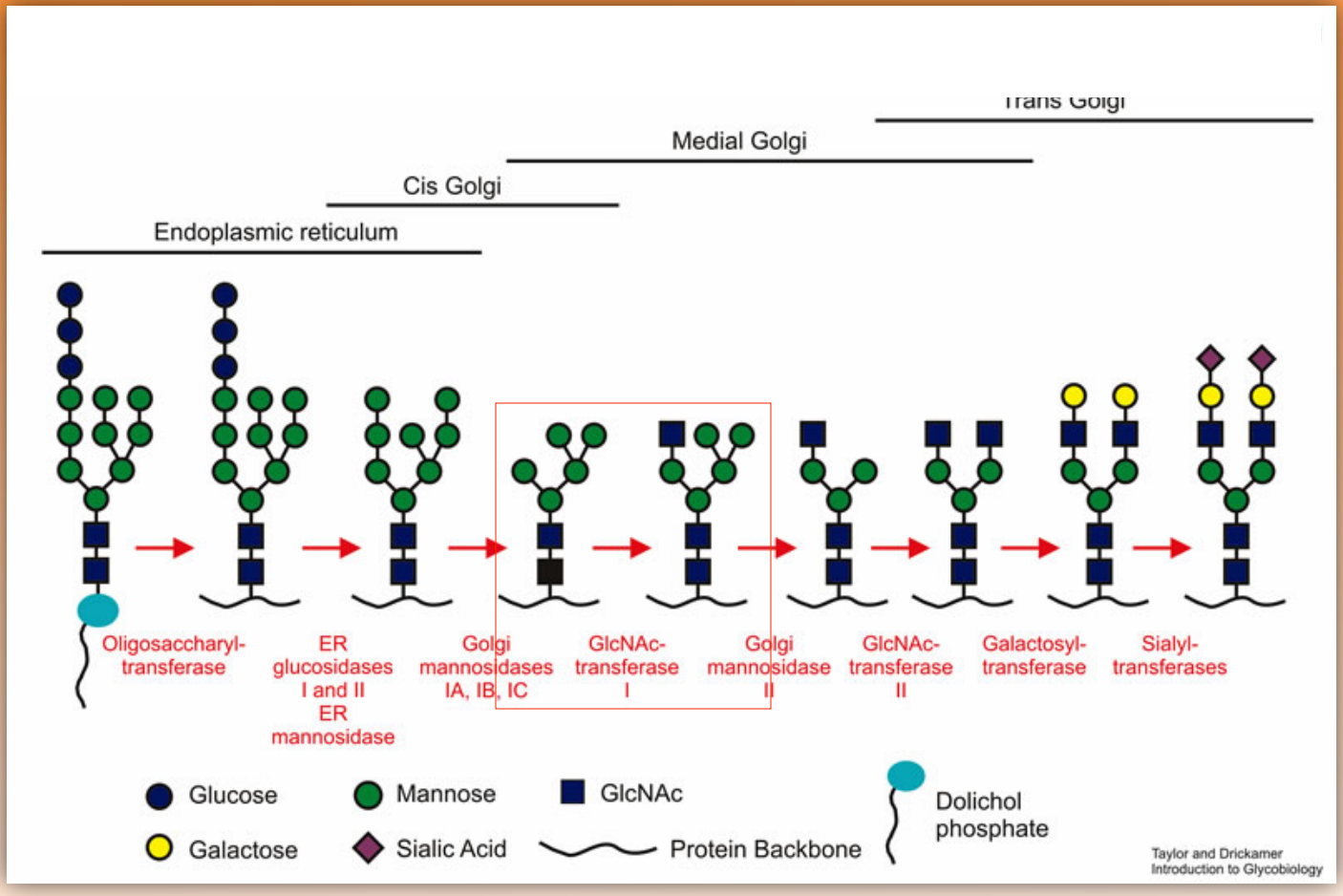


Pathway of dolichol-PP-oligosaccharide synthesis



A summary

Processing: High-mannose glycan to complex glycan



The GlcNAc transferases of the medial Golgi

- **GlcNAc transferase I**: adds a GlcNAc residue to the 1,3-arm of the trimmed *N*-glycan core
- **GlcNAc transferase II**: adds a GlcNAc residue to the 1,6-arm of the maturing *N*-glycan