N-Glycans

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N-glycans

- N-glycans are covalently attached to protein at asparagine (Asn) residues by an N-glycosidic bond
- Five different N-glycan linkages are known, of which N-acetylglucosamine to asparagine (GlcNAc 1-Asn) is the most common
- Asn-X-Ser/Thr “sequons” in a protein are candidates for receiving an N-glycan
- Complicated biosynthesis
- Dolichol phosphate (Dol-P) as carrier for N-glycan biosynthesis
- N-glycan synthetic pathway is conserved in all of the metazoa, in plants, and in yeast
- Other linkages to Asn: glucose, N-acetylgalactosamine (GalNAc), rhamnose. And linkage to argnine: glucose
- N-glycans affect many properties of glycoproteins including their conformation, solubility, antigenicity, and recognition by glycan-binding proteins.
- Defects in N-glycan synthesis lead to a variety of human diseases

Types of N-glycans

Types of N-glycans. N-glycans added to protein at Asn-X-Ser/Thr sequons are of three general types in a mature glycoprotein: oligomannose, complex, and hybrid. Each N-glycan contains the common core Man" 1–6(Man" 1–3)Man 1–4GlcNAc 1–4GlcNAc 1–Asn (Man3GlcNAc2Asn).
N-glycan Sites

- N-glycans occurs only on the Asn-X-Ser/Thr sequon
- About two thirds of protein contain the Asn-X-Ser/Thr consensus sequence. Among which more than two thirds of those sequons are likely to be N-glycosylated
- when Asn-X-Ser/Thr sequons are present in a deduced amino acid sequence encoded by a cDNA, they are not identified categorically as N-glycan sites, but are referred to as potential N-glycan sites. Proof that an N-glycan is actually present at a potential site requires experimental evidence
- Occasionally, N-glycans occurs at Asn-X-Cys
- The transfer of N-glycans to Asn-X-Ser/Thr sequons occurs on the lumenal side of the endoplasmic reticulum (ER) membrane while the protein moiety is being synthesized on ER-bound ribosomes and is translocating through the translocon in the ER membrane

N-glycan Isolation & Analysis

- Release
  - Peptide-N-glycosidase F (PNGase F): remove oligomannose, hybrid and complex N-glycan from ASN, but N-glycan core needs not to be modified.
  - PNGase A: remove all N-glycan from Asn
  - Endoglycosidase H: release oligomannose and hybrid N-glycans, but not complex N-glycans.
  - Endoglycosidase F: release simple biantennary N-glycans, but not oligomannose or hybrid N-glycans
  - Hydrazinolysis
  - Protease
- Purification and analysis
  - ion-exchange and size-exclusion chromatography, high-pressure liquid chromatography (HPLC) methods, and affinity chromatograph
  - composition, linkage and sequence
**Synthesis of N-glycan**

I. Synthesis of the Dolichol-P-P-Glycan Precursor

II. Transfer of the Dolichol-linked Precursor to Nascent Proteins

III. Early Processing Steps: \( \text{Glc}_3\text{Man}_9\text{GlcNAc}_2\text{Asn} \) to \( \text{Man}_9\text{GlcNAc}_2\text{Asn} \)

IV. Late Processing Steps: From \( \text{Man}_9\text{GlcNAc}_2\text{Asn} \) to Hybrid and Complex N-Glycans

V. Maturation of N-Glycans

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**Dolichol Phosphate**

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\text{CH}_3\text{C}=\text{CH}-\text{CH}_2-(\text{CH}_2\text{-C}=\text{CH}-\text{CH}_2)_n\text{-CH}_2\text{-CH}-\text{CH}_2\text{-CH}_2\text{-O-PO}_3^-\text{O}^- \\
\text{CH}_3\text{C}=\text{CH}-\text{CH}_2-(\text{CH}_2\text{-C}=\text{CH}-\text{CH}_2)_n\text{-CH}_2\text{-CH}-\text{CH}_2\text{-CH}_2\text{-O-PO}_3^-\text{O}^- \\
\text{CH}_3\text{C}=\text{CH}-\text{CH}_2-(\text{CH}_2\text{-C}=\text{CH}-\text{CH}_2)_n\text{-CH}_2\text{-CH}-\text{CH}_2\text{-CH}_2\text{-O-PO}_3^-\text{O}^-
\]

Dolichol is a polyisoprenol lipid comprised of five-carbon isoprene units linked linearly in a head-to-tail fashion.

The number of isoprene units in dolichol varies within cells and between cell types and organisms.

Dol-P is used in N-glycan synthesis

N-Glycan synthesis begins by the transfer of GlcNAc-1-P from UDP-GlcNAc to Dol-P to generate dolichol pyrophosphate \( N\)-acetylglucosamine (Dol-P-P-GlcNAc). This reaction is inhibited by tunicamycin.
Dolichol (red squiggle) phosphate (Dol-P) located on the cytoplasmic face of the ER membrane receives GlcNAc-1-P from UDP-GlcNAc in the cytoplasm to generate Dol-P-P-GlcNAc. Dol-P-P-GlcNAc is extended to Dol-P-P-GlcNAc-Man₅, using GDP-Man as precursor before being “flipped” across the ER membrane to the luminal side. On the luminal face of the ER membrane, four mannose residues are added from Dol-P-Man and three glucose residues from Dol-P-Glc. Dol-P-Man and Dol-P-Glc are also made on the cytoplasmic face of the ER and “flipped” onto the luminal face. Yeast mutants defective in an ALG gene have been used to identify the gene that encodes the enzyme responsible for each transfer. Some reactions affected in congenital disorders of glycosylation (CDG) are noted.

**Processing and maturation of an N-glycan**

The mature Dol-P-P-glycan is transferred to Asn-X-Ser/Thr sequons during protein synthesis as proteins are being translocated into the ER. Following transfer of the 14-sugar Glc-Man₅GlcNAc₂ glycan to protein, glucosidase_I in the ER remove the three glucose residues, and ER mannosidase removes a mannose residue. These reactions are intimately associated with the folding of the glycoprotein assisted by the lectin calnexin and calreticulin, and they determine whether the glycoprotein continues to the Golgi or is degraded. Another lectin, termed EDEM, binds to mannose residues on misfolded glycoproteins and escorts them via retrotranslocation into the cytoplasm for degradation. The removal of the first glucose (and therefore all glucose) can be blocked by castanospermin. For most glycoproteins, additional mannose residues are removed in the cis compartment of the Golgi until Man₃GlcNAc₂Asn is generated. The mannosidase inhibitor deoxymannojirimycin blocks the removal of these mannose residues. The action of GlcNAcT-1 on Man₃GlcNAc₂Asn in the medial-Golgi initiates the first branch of an N-glycan. This reaction is blocked in the Lec1 CHO mutant in which GlcNAcT-1 is inactive, leaving Man₃GlcNAc₂Asn, which is not further processed. "Mannosidase II removes two outer mannose residues in a reaction that is blocked by the inhibitor swainsonine. The action of alpha-mannosidase II generates the substrate for GlcNAcT-II. The resulting biantenary N-glycan is extended by the addition of fucose, galactose, and sialic acid to generate a complex N-glycan with two branches. The addition of galactose does not occur in the Leã8 CHO mutant, which has an inactive UDP-Gal transporter. In Leã8 mutants, complex N-glycans terminate in N-acetylglucosamine. The addition of sialic acid does not occur in the Lec2 CHO mutant, which has an inactive CMP-sialic acid transporter. In Lec2 mutants, complex N-glycans terminate with galactose. Complex N-glycans can have many more sugars than shown in this figure, including additional residues attached to the core, additional branches, branches extended with poly-N-acetyllactosamine units, and different “capping” structures. Also shown is the special case of lysozyme hydrolyses that acquire a GlcNAc-1-P at C-6 of mannose residues on oligomannose N-glycans in the cis-Golgi. The N-acetylglucosamine is removed in the trans-Golgi by a glycosidase, thereby exposing Man-6-P residues that are recognized by a Man-6-P receptor and routed to an acidified, prelysosomal compartment.
Related Enzymes, Inhibitors and N-glycans in Yeast

- Oligosaccharyltransferase (Ost)
  - Bind to the Glc$_3$Man$_9$GlcNAc$_2$-P-P-Dol and transfer the glycan to Asn-X-Ser/Thr in newly synthesized region of proteins by cleavage of the high energy GlcNAc-P bond, releasing Dol-P-P.
  - A multisubunit protein complex in the ER membrane: Ost1p - Ost6p, Wbp1p, Wsp1p and Stt3p. Stt3p appears the catalytic domain

- Tunicamycin: a analog of GlcNAc and inhibits GlcNAc-1-phosphotransferase
- Castanospermine and deoxynojirimycin: Glucosidase I inhibitor
- Swainsonine: inhibits $\alpha$-Mannosidase II
- Deoxymannojirimycin: $\alpha$-mannosidase 1

- Yeast add mannose: Do not truncate the Man$_8$GlcNAc$_2$ N-glycans that enters cis-Golgi, and add additional Man residues to Man$_8$GlcNAc$_2$ to produce oligomannose structures containing many branched mannose resides. Such large yeast mannans are antigenic in humans. Therefore, yeast is not a good host for the production of recombinant therapeutically glycoproteins.

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**Synthesis of dolichol-P-P-GlcNAc$_2$Man$_9$Glc$_3$**

Dolichol (red squiggle) phosphate (Dol-P) located on the cytoplasmic face of the ER membrane receives GlcNAc-1-P from UDP-GlcNAc in the cytoplasm to generate Dol-P-P-GlcNAc. Dol-P-P-GlcNAc is extended to Dol-P-P-GlcNAc$_2$Man$_9$Glc$_3$ using GDP-Man as precursor before being “flipped” across the ER membrane to the lumenal side. On the luminal face of the ER membrane, four mannose residues are added from Dol-P-Man and three glucose residues from Dol-P-Glc. Dol-P-Man and Dol-P-Glc are also made on the cytoplasmic face of the ER and “flipped” onto the luminal face. Yeast mutants defective in an ALG gene have been used to identify the gene that encodes the enzyme responsible for each transfer. Some reactions affected in congenital disorders of glycosylation (CDG) are noted.
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Branching of complex N-glycans

The hybrid and mature, biantennary, complex N-glycans may contain more branches due to the action of branching N-acetylgalcosaminyltransferases in the Golgi. The latter can act only after the prior action of GlcNAcT-I. GlcNAcT-III transfers N-acetylgalcosamine to the β-linked mannos residue in the core to generate the bisecting N-acetylgalcosamine. The presence of this residue inhibits the action of α-mannosidase II, thereby generating hybrid structures. A biantennary N-glycan may also accept the bisecting N-acetylgalcosamine. More highly branched N-glycans are generated by the action of GlcNAcT-IV, GlcNAcT-V, and GlcNAcT-VI and may also carry the bisecting N-acetylgalcosamine. The most highly branched structures with seven N-acetylgalcosamine residues (including the bisecting N-acetylgalcosamine) on the core of N-glycans have been found in a bird glycoprotein. Each N-acetylgalcosamine branch may be elongated with galactose, poly-N-acetylactosamine, sialic acid, and fucose.
Elongation of branch N-acetylglucosamine residues of N-glycans

(A) A single N-acetyllactosamine unit is generated when galactose is transferred to a branch N-acetylglucosamine on an N-glycan (R). Further elongation to form poly-N-acetyllactosamine occurs by sequential addition of galactose and N-acetylglucosamine, as shown. This structure is composed of type-2 poly-N-acetyllactosamine units. (B) Type-1 N-acetyllactosamine units can also be present in poly-N-acetyllactosamine. (C) Transfer of N-acetylgalactosamine to N-acetylglucosamine generates LacdiNAc.

Modifications of the core of N-glycans

(A) A fucose residue may be transferred to the core of N-glycans after GlcNAcT-I has acted. Thus, hybrid and biantennary, complex N-glycans may have a core fucose. (B) Plants and invertebrates may have additional modifications to the core, with fucose on either N-acetylglucosamine residue (or both for invertebrates) and a xylose attached to the core β-linked mannose residue of plant N-glycans. (C) Other additions to the core have been detected in mammalian cells.
Typical complex N-glycan structures found on mature glycoproteins

Transferases & Transporters in N-Glycan Biosynthesis

- The glycosyltransferases that operate in the ER: multitransmembrane proteins
- Glycosyltransferases in Golgi: type II membrane proteins with a small cytoplasmic amino-terminal domain, a single transmembrane domain, and a large lumenal domain that has an elongated stem region and a globular catalytic domain. The stem region can be cleaved, releasing the catalytic domain into the lumen of the Golgi and allowing its secretion
- Nucleotide sugar transporters: a multitransmembrane protein and usually contains ten membrane-spanning domains. Nucleotide sugars are synthesized in the cytoplasm, except for CMP-sialic acid, which is synthesized in the nucleus. They are subsequently concentrated in the appropriate compartment following transport across the membrane by specialized nucleotide sugar transporters
N-LINKED GLYCOPROTEINS COMPRIZE MANY GLYCOFORMS

- A homogeneous glycoprotein component of a population is called a glycoform.
- Content of N-glycans
- A range of different N-glycans on a particular Asn-X-Ser/Thr N-glycosylation sequon
  - mouse zona pellucida glycoprotein: 58 different complex N-glycan
- Site-specific glycan heterogeneity (microheterogeneity)
- Protein sequence or conformation can cause N-glycan diversity, presumably by affecting substrate availability for Golgi glycosidasases or glycosyltransferases
- Affecting factors
  - Nucleotide sugar metabolism, transport rates of the glycoprotein through the lumen of the ER and Golgi, and the proximity of an N-glycan attachment sequon to a transmembrane domain
  - Localization of glycosyltransferases within subcompartments of the Golgi
  - Most glycosyltransferases and glycosidasases require the prior actions of other glycosyltransferases and glycosidasases before they can carry out their reactions.

Functions of N-Glycans

- Study approaches
  - N-glycosylation inhibitors (Tunicamycin, castanospermine, deoxyxojirimycin, swainsonine)
  - Mutants
  - Human diseases that arise from a defect in N-glycan synthesis [congenital disorders of glycosylation (CDG)]
- Function
  - Retaining growth factor and cytokine receptors at the cell surface
  - Regulates immunity, neuronal cell migration, and contributes to emphysema of the lung and inflammation
  - Mediate cell–cell interactions important for leukocyte extravasation from the blood stream and regulate lymphocyte homing to lymph nodes
  - Become more branched when cells become cancerous, and this change facilitates cancer progression