



## Biosynthesis of N and O Glycans

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# Biosynthesis of N- and O-Glycans

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Protein-bound oligosaccharides are linked to the polypeptide core through a variety of different linkages. The two most diverse mammalian glycoprotein classes are the N- and O-glycans in which the linkages are asparagine-N-acetyl-D-glucosamine (Asn-GlcNAc) and serine (threonine)-N-acetyl-D-galactosamine (Ser(Thr)-GalNAc), respectively.

## N-GLYCAN STRUCTURE

All N-glycans share the common core structure  $\text{Man}\alpha 1-6(\text{Man}\alpha 1-3)\text{Man}\beta 1-4\text{GlcNAc}\beta 1-4\text{GlcNAc}\beta\text{-Asn}$ . There is, however, an enormous variety and complexity in the oligosaccharides attached to this core. At least four groups are recognized<sup>(1)</sup>:

- (i) High mannose N-glycans contain only D-mannose (Man) residues attached to the core.
- (ii) Complex N-glycans have “antennae” or branches attached to the core. These antennae are initiated by the action of four mammalian GlcNAc-transferases<sup>(2,3)</sup> (designated GlcNAc-transferases I, II, IV and V, Figure 1) and may be further elongated by the addition of D-galactose, N-acetyl-D-galactosamine, L-fucose, sialic acid and sulfate. The number of antennae in mammals ranges from two (biantennary) to four (tetraantennary) but hen oviduct can make a pentaantennary structure due to the action of GlcNAc-transferase VI.
- (iii) Hybrid N-glycans have only Man residues on the  $\text{Man}\alpha 1-6$  arm of the core and one or two antennae on the  $\text{Man}\alpha 1-3$  arm.
- (iv) Poly-N-acetylglucosamine N-glycans contain repeating units of  $(\text{Gal}\beta 1-4\text{GlcNAc}\beta 1-3-)$  attached to the core. This repeating structure may be branched, due to the action of a  $\beta 6$ -GlcNAc-transferase, to form the  $\text{Gal}\beta 1-4\text{GlcNAc}\beta 1-6(\text{Gal}\beta 1-4\text{GlcNAc}\beta 1-3)\text{Gal-}$  structure. All N-glycans except the high mannose type may be “bisected” by a GlcNAc residue attached in  $\beta 1-4$  linkage to the  $\beta$ -linked Man of the core due to the action of GlcNAc-transferase III. The Asn-linked GlcNAc of the core of all N-glycans except the high mannose type may have an  $\alpha 1-6$ -linked Fuc (or  $\alpha 1-3$  in plants).

## O-GLYCAN STRUCTURE

O-glycans are found as:

- the monosaccharide  $\text{GalNAc}\alpha\text{-Ser(Thr)}$ ,
- disaccharides such as  $\text{sialyl}\alpha 2-6\text{GalNAc}\alpha\text{-Ser(Thr)}$  or  $\text{Gal}\beta 1-3\text{GalNAc}\alpha\text{-Ser(Thr)}$ , and
- larger glycans with three distinct regions (core, backbone, non-reducing terminus). There are at least six O-glycan core structures<sup>(4)</sup>:

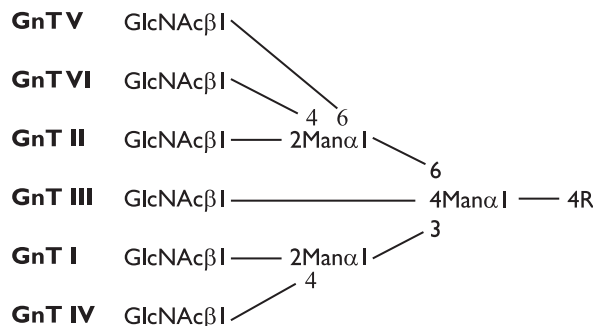


Figure 1. The “branching” GlcNAc-transferases. Five antennae can be initiated on the  $\text{Man}\alpha 1-6(\text{Man}\alpha 1-3)\text{Man}\beta 1-4\text{GlcNAc}\beta 1-4\text{GlcNAc}\beta\text{-Asn}$  core of N-glycans by the actions of GlcNAc-transferases I, II, IV, V and VI. A “bisecting” GlcNAc can be added by GlcNAc-transferase III.

Core 1:  $\text{Gal}\beta 1-3\text{GalNAc-R}$ ;

Core 2:  $\text{GlcNAc}\beta 1-6(\text{Gal}\beta 1-3)\text{GalNAc-R}$ ;

Core 3:  $\text{GlcNAc}\beta 1-3\text{GalNAc-R}$ ;

Core 4:  $\text{GlcNAc}\beta 1-6(\text{GlcNAc}\beta 1-3)\text{GalNAc-R}$ ;

Core 5:  $\text{GalNAc}\alpha 1-3\text{GalNAc-R}$ ;

Core 6:  $\text{GlcNAc}\beta 1-6\text{GalNAc-R}$  (R is  $\alpha\text{-Ser/Thr}$ ).

These cores can be elongated to form the backbone region by addition of Gal in  $\beta 1-3$  and  $\beta 1-4$  linkages, and GlcNAc in  $\beta 1-3$  and  $\beta 1-6$  linkages. The termini are formed by the addition of D-galactose, N-acetyl-D-galactosamine, L-fucose, sialic acid and sulfate. Recently, other unusual modifications have been reported.

## BIOSYNTHESIS OF N-GLYCANS

The biosynthesis of N-glycans<sup>(1,5)</sup> begins in the rough endoplasmic reticulum with the co-translational transfer of a large oligosaccharide ( $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$ ) from dolichol pyrophosphate oligosaccharide to an Asn residue in the polypeptide (step 1, Figure 2). The Asn must be in an Asn-Xaa-Ser/Thr triplet known as a “sequon” (where Xaa is any amino acid except Pro)<sup>(6)</sup>. This is followed by the removal of three glucose and four mannose residues within the lumen of the endoplasmic reticulum and Golgi apparatus due to the processing actions of specific  $\alpha$ -glucosidases and  $\alpha$ -mannosidases (steps 2 to 5, Figure 2). The product of this processing is the structure  $[\text{Man}\alpha 1-6(\text{Man}\alpha 1-3)\text{Man}\alpha 1-6](\text{Man}\alpha 1-3)\text{Man}\beta 1-4\text{GlcNAc}\beta 1-4\text{GlcNAc}\beta\text{-Asn}$  ( $\text{Man}_5\text{GlcNAc}_2\text{-R}$ ) which is the starting point for the synthesis of all complex and hybrid N-glycans.

The key enzyme for the conversion of high-mannose to complex and hybrid N-glycans is GlcNAc-transferase I

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(step 6, Figure 2) which adds a GlcNAc in  $\beta$ 1-2 linkage to the  $\text{Man}\alpha$ 1-3 $\text{Man}\beta$ 1-4 $\text{GlcNAc}\beta$ - arm of the core. The presence of a  $\beta$ 2-linked GlcNAc residue at the non-reducing terminus of this arm is essential for the subsequent actions of several enzymes in the processing pathway (2,3,7), i.e.  $\alpha$ 3/6-mannosidase II (step 7, Figure 2), GlcNAc-transferase II (step 8, Figure 2), core  $\alpha$ 6-fucosyltransferase (step 9, Figure 2) and GlcNAc-transferases III and IV (Figure 1). GlcNAc-transferase I is therefore a “go” signal for all these enzymes. Similarly, GlcNAc-transferase V needs the prior action of GlcNAc-transferase II.

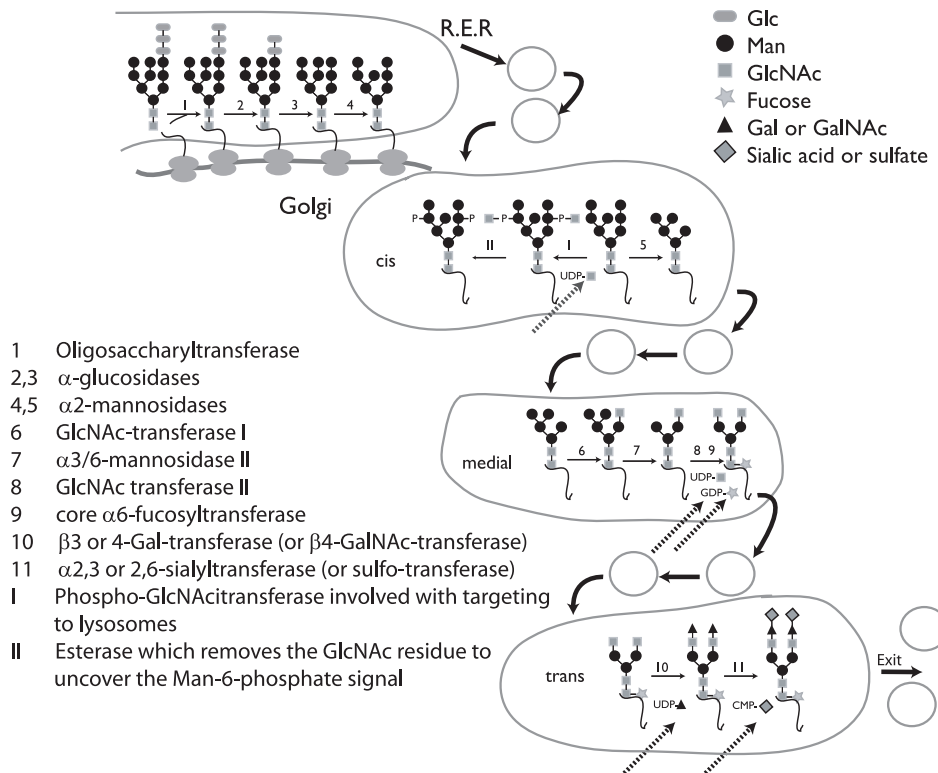
There are many cross-roads during biosynthesis at which more than one enzyme competes for a common substrate. The route taken by the synthetic pathway at a competition point is dictated primarily by the relative activities of the competing transferases. Some glycosyl residues serve as a “stop” signal in the synthetic pathway, e.g., insertion of a bisecting GlcNAc prevents the actions of  $\alpha$ 3/6-mannosidase II, core  $\alpha$ 6-fucosyltransferase, and of GlcNAc-transferases II, IV and V<sup>(2,8)</sup> thereby effectively halting further branching. Although this reaction halts branching in the medial Golgi cisternae, it does not prevent movement to the trans-Golgi followed by addition of D-galactose or N-acetyl-D-galac-

tosamine (step 10, Figure 2), sialic acid or sulfate (step 11, Figure 2) or other residues (e.g. L-fucose) to the antennae.

## BIOSYNTHESIS OF O-GLYCANS

Figure 3 shows some of the enzymes involved in the synthesis and elongation of O-glycan cores 1 to 4. As is the case for the synthesis of N-glycans, the synthetic paths for O-glycans tend to be ordered rather than random, i.e., certain key glycosyl residues either divert the synthetic flux away from or into a particular pathway. For example, the orders of synthesis of cores 2 and 4 are, respectively, GalNAc-R to Gal $\beta$ 1-3GalNAc-R to GlcNAc $\beta$ 1-6(Gal $\beta$ 1-3)GalNAc-R (Figure 3, reactions 1 and 2), and GalNAc-R to GlcNAc $\beta$ 1-3GalNAc-R to GlcNAc $\beta$ 1-6(GlcNAc $\beta$ 1-3)GalNAc-R (Figure 3, reactions 3 and 4). Once the core 1 structure has been elongated, the synthesis of the core 2 analogs becomes much less likely; elongation (Figure 3, reaction 8) prevents the action of core 2  $\beta$ 6-GlcNAc-transferase (Figure 3, reaction 13). The structure GlcNAc $\beta$ 1-6GalNAc-Ser(Thr) has been reported on several human glycoproteins suggesting that human tissues may contain a  $\beta$ 6-GlcNAc-transferase which acts directly on GalNAc-R (Figure 3, reaction 5).

Figure 2. Biosynthetic scheme for N-glycans showing the enzymatic steps involved in their synthesis. The genes for the enzymes catalyzing steps 1, 4-8, 10 ( $\beta$ 4-Gal-transferase) and 11, (the sialyltransferases) have been cloned (9-14).



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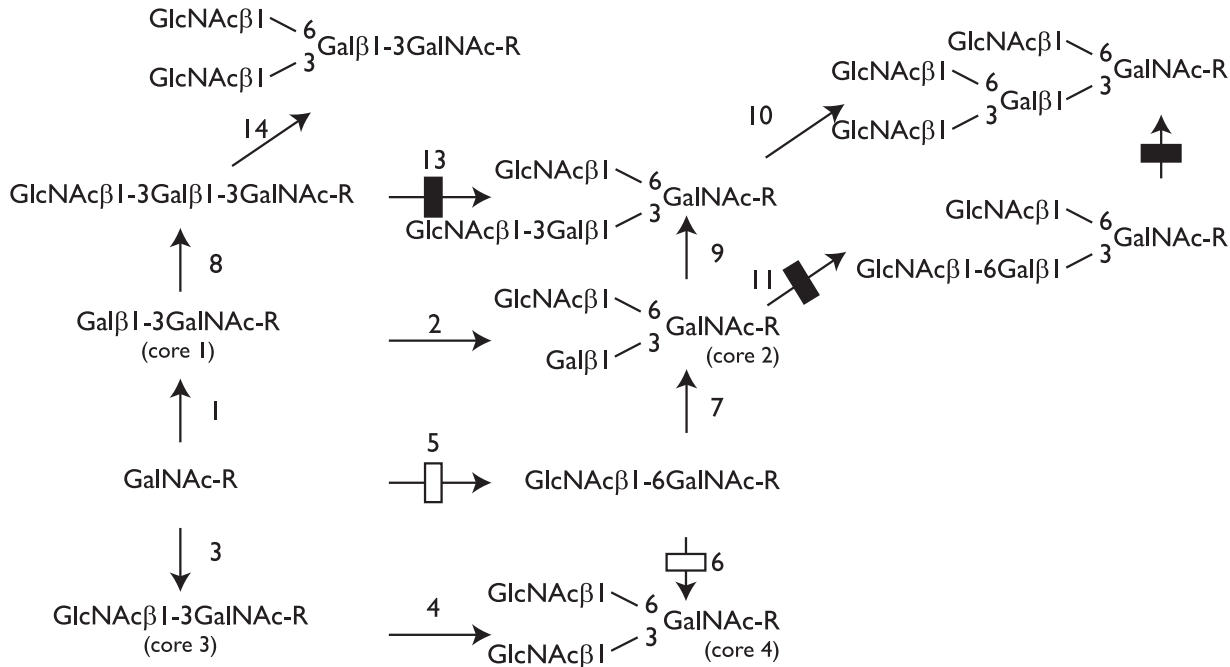


Figure 3. Biosynthetic scheme for O-glycans. Assembly of Ser(Thr)-GalNAc oligosaccharides, showing synthesis of the four core classes and some commonly occurring derivatives of core classes 1 and 2<sup>(3,4)</sup>. Arrows blocked with filled rectangles (reactions 11, 12 and 13) indicate reactions that do not take place. The conversion of GalNAc-R to GlcNAc $\beta$ 1-6GalNAc-R (reaction 5) has been reported in human ovarian tissue. The conversion of GlcNAc $\beta$ 1-6GalNAc-R to core 4 (reaction 6) can occur but is very slow. The gene for the core 2 GlcNAc-transferase has been cloned<sup>(15)</sup>.



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