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# Oligosaccharide Branching of Glycoproteins: Biosynthetic Mechanisms and Possible Biological Functions

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*Phil. Trans. R. Soc. Lond. B* 1982 **300**, 145-159
doi: 10.1098/rstb.1982.0162

*Phil. Trans. R. Soc. Lond.* B **300**, 145–159 (1982)
*Printed in Great Britain*



# Oligosaccharide branching of glycoproteins: biosynthetic mechanisms and possible biological functions

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One of the most striking features of *N*- and *O*-glycosyl oligosaccharides and of lipid-linked oligosaccharides is the high degree of branching of these complex structures. Both proteins and nucleic acids are essentially linear structures and are synthesized by template mechanisms. The branched nature of complex carbohydrates dictates a totally different mechanism of biosynthetic control. Although there are undoubtedly many factors controlling this assembly (e.g. subcellular compartmentation, availability of substrates, cations), our laboratory has studied primarily the enzymatic factors that control the assembly of branched *N*-glycosyl (Asn-GlcNAc type) and *O*-glycosyl (Ser[Thr]-GalNAc type) oligosaccharides. There are three basic types of control points that appear to direct biosynthesis. (*a*) There may be two or more enzymes capable of acting on a single common substrate. Control at this juncture is exerted by the relative activities of these enzymes in a particular tissue. (*b*) Addition of a specific sugar to the growing oligosaccharide may shut off one or more subsequent enzyme steps, thereby 'freezing' the structure at a certain stage in its synthesis. (*c*) Progression of the pathway may be impossible until a certain key sugar residue is inserted into the growing oligosaccharide chain. Examples of all three types of control occur in the assembly of both *N*- and *O*-glycosyl oligosaccharides. This paper discusses our work on the *N*-acetylglucosaminyltransferases, which initiate branches in *N*-glycosyl oligosaccharides, as well as some studies on glycosyltransferases that control the assembly of the four basic Ser(Thr)-GalNAc cores. Important features at all stages of control are the three-dimensional shape of the oligosaccharide, the effect of certain key sugar residues on this three-dimensional shape and the stereochemistry of the interaction of oligosaccharides with proteins. From a functional point of view, protein–oligosaccharide interaction is of vital importance not only to enzyme control mechanisms but to a variety of biological problems such as malignancy and cell–cell interactions, differentiation and development, and susceptibility of cells to hormones, drugs and toxins.

## Introduction

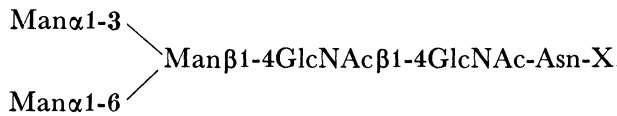
One of the most consistent biochemical correlations with malignant transformation is a relative increase in the release of high molecular mass sialic acid-rich glycopeptide material from the cell surface by proteolytic cleavage (Warren *et al.* 1978). Ogata *et al.* (1976) first suggested that this material contained highly branched (tri- and tetra-antennary) Asn-linked oligosaccharides. Proof for this hypothesis was obtained by Takasaki *et al.* (1980), who found that transformation of BHK cells with polyoma virus led to a relative decrease in bi-antennary oligosaccharides, a relative increase in tetra-antennary oligosaccharides and the appearance of novel penta- and hexa-antennary structures. However, the correlation between degree of branching and malignant transformation has exceptions (Warren *et al.* 1978; Rachesky *et al.* 1982; Cossu *et al.* 1982). Our laboratory has for several years studied the enzymic mechanisms responsible for the

branching of both *N*-glycosyl and *O*-glycosyl oligosaccharides. This topic is of interest not only because of the possible association of branched structures with malignancy but also because the degree of branching varies in other situations and the functional significance of these variations remains obscure.

## Structural considerations

### *Asn-GlcNAc oligosaccharides*

Asn-GlcNAc oligosaccharides appear to have a common core:




Relatively primitive organisms (e.g. yeasts) have only the 'high-mannose' type of structure, which contains a large number of Man residues attached to this common core. More advanced organisms (e.g. birds and mammals) have retained this high-mannose structure (although with far fewer Man residues than are found in yeasts) and have developed in addition a more 'complex' series of structures. There are now at least ten classes of *N*-glycosyl oligosaccharides (Carver & Grey 1981; Turco *et al.* 1980) and more may eventually be discovered. These classes are: bi-antennary complex (I), tri-antennary complex (II), tetra-antennary complex (III), bisected bi-antennary complex (IV), bisected tri-antennary complex (V), high mannose (VI), bi-antennary hybrid (VII), bisected bi-antennary hybrid (VIII), bisected tri-antennary hybrid (IX) and polylactosaminoglycan or erythroglycan (X). Figure 1 shows examples of some of these structures.

### *Ser(Thr)-GalNAc oligosaccharides*

Ser(Thr)-GalNAc oligosaccharides are even more diverse than the *N*-glycosyl oligosaccharides. The simplest structures (found, for example, in ovine submaxillary mucin) are GalNAc-Ser(Thr)-X and sialylα2-6GalNAc-Ser(Thr)-X. More complex structures have recently been classified into four core types (Schachter & Williams 1982):

type 1 core, Galβ1-3GalNAc-Ser(Thr)-X;

type 2 core, Galβ1-3(GlcNAcβ1-6)GalNAc-Ser(Thr)-X;

type 3 core, GlcNAcβ1-3GalNAc-Ser(Thr)-X;

type 4 core, GlcNAcβ1-3(GlcNAcβ1-6)GalNAc-Ser(Thr)-X.

These cores can then be elongated into a variety of different branched structures, some of which carry human blood group antigenic determinants such as A, B, H, Lewis$^a$, Lewis$^b$ and Ii.

## Biosynthesis of Asn-GlcNAc high-mannose structures

The preceding papers have dealt in detail with the early steps in the assembly of *N*-glycosyl oligosaccharides. Biosynthesis begins within the rough endoplasmic reticulum with transfer of $(Glc)_3(Man)_9(GlcNAc)_2$ from dolichyl pyrophosphate-oligosaccharide to nascent polypeptide. Processing within the rough endoplasmic reticulum removes the 3Glc residues; this is followed

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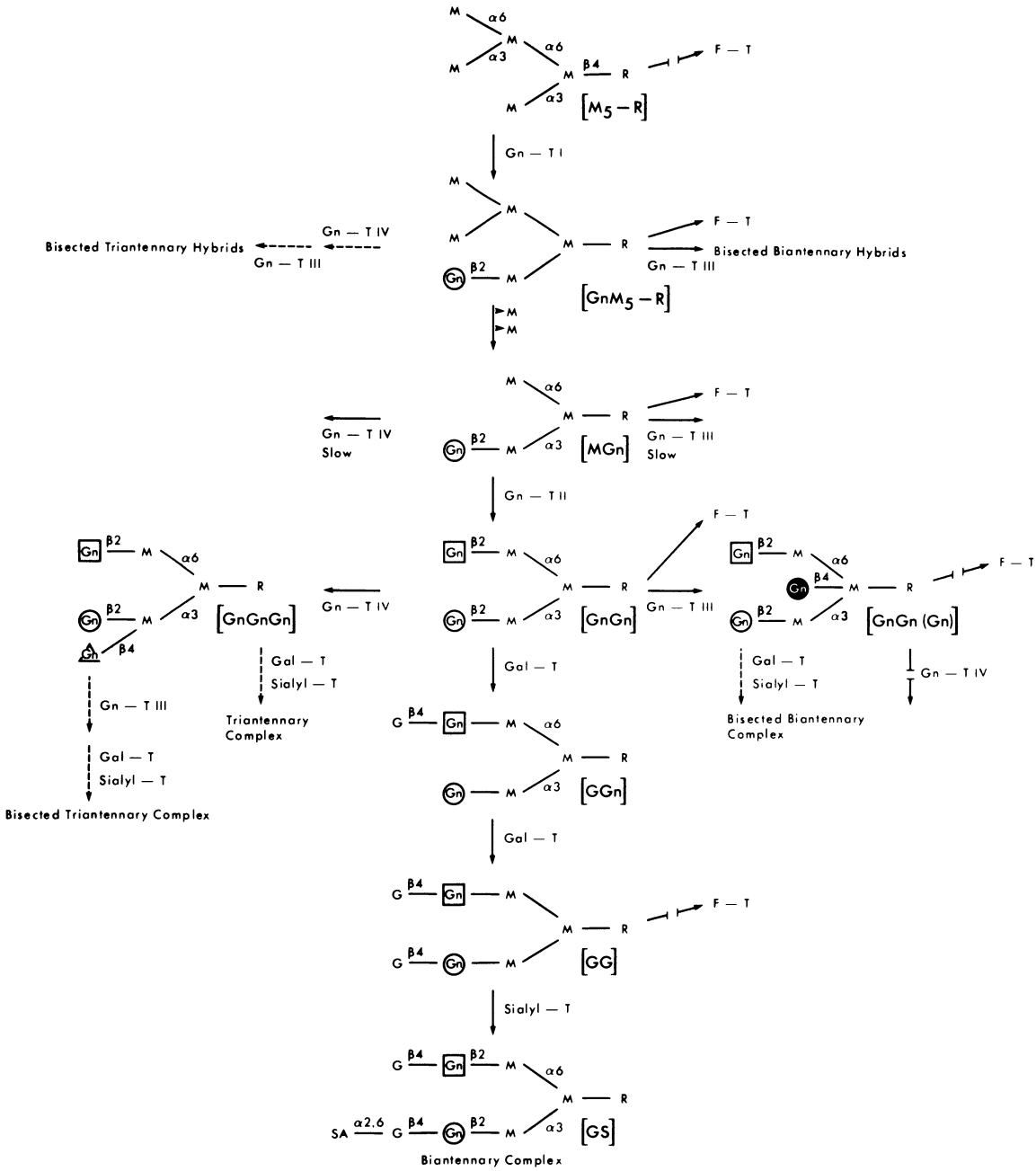


FIGURE 1. Golgi-localized pathway for the assembly of *N*-glycosyl hybrid and complex oligosaccharides. The pathway is discussed in detail in the text. Hybrid oligosaccharides carry Man residues on the Manα1-6- arm of the core and antennae (usually GlcNAc- or Galβ1-4GlcNAc-) on the Manα1-3- arm. Hybrid oligosaccharides may be non-bisected (like structure $GnM_5$-R) but are usually bisected by a GlcNAc residue linked β1-4- to the β-linked Man residue. Abbreviations: M, Man; Gn, GlcNAc; F, Fuc; G, Gal; SA, sialyl; R, GlcNAcβ1-4GlcNAc-Asn-X; T, transferase. Glycopeptide nomenclature: complex oligosaccharides containing only 3Man residues are named according to the sugars at the non-reducing termini of the antennae; the sugar on the Manα1-6- arm is named first. Order of sugar addition: the order of GlcNAc addition is discussed in the text. Immunoglobulins isolated from human multiple myeloma serum have been shown to carry the structures GGn and GS (rather than GnG and SG) suggesting that Gal-T prefers the Manα1-6- arm and sialyl-T prefers the Manα1-3- arm. Enzymic evidence in support of this ordered addition has been obtained (Van den Eijnden *et al.* 1980; Rao & Mendicino 1978).




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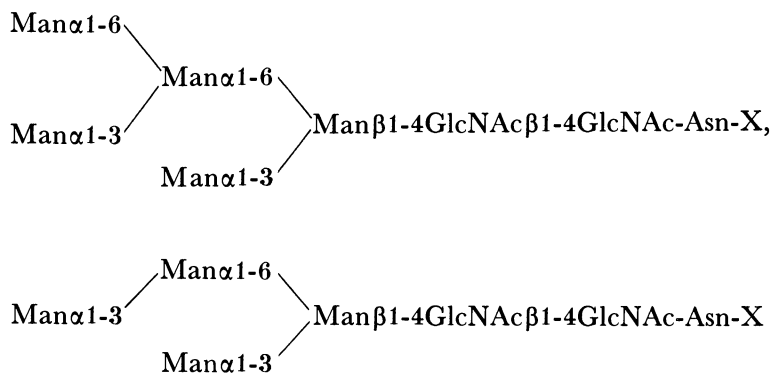
by the removal, partly in the rough endoplasmic reticulum but mainly within the Golgi apparatus, of four α-2-linked Man residues, to yield a key intermediate (shown at the top of figure 1) containing 5Man residues and 2GlcNAc residues. This structure and a similar compound containing only 4Man residues are the smallest high-mannose structures found in avian and mammalian glycoproteins. The largest high-mannose structure found in higher organisms contains 9Man residues, although yeast glycoproteins may contain much larger oligosaccharides. For some protein-bound oligosaccharides, the synthetic path goes no further than the high-mannose stage. For others, unknown factors (possibly the amino acid sequence near the relevant Asn residue) direct the synthetic path towards complex oligosaccharides (see below).

## Biosynthesis of Asn-GlcNAc complex and hybrid structures

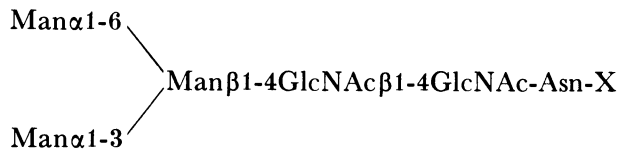
### *UDP-GlcNAc:* α-D-*mannoside* β2-*GlcNAc-transferase I*

Complex and hybrid structures occur only in higher organisms, and the synthesis of these compounds is preceded by the synthesis of high-mannose structures, i.e. the synthetic pathway recapitulates phylogeny. The high-mannose structures are processed by a series of α-glucosidases and α-mannosidases to the intermediate shown at the top of figure 1. This intermediate is the precursor of complex and hybrid oligosaccharides. It is acted upon by UDP-GlcNAc: α-D-mannoside β2-GlcNAc-transferase I (Gn-T I, figure 1), an enzyme that initiates the first branch or antenna by inserting a GlcNAc residue in β1-2 linkage on the terminal Manα1-3-residue. Gn-T I was first shown to be different from an analogous enzyme (UDP-GlcNAc: α-D-mannoside β2-GlcNAc-transferase II, Gn-T II) by the isolation of lectin-resistant mutants of Chinese hamster ovary cells deficient in Gn-T I (Stanley 1980; Narasimhan *et al.* 1977; Gottlieb *et al.* 1974, 1975). When such Gn-T I-deficient cells are infected with vesicular stomatitis virus, the viral envelope glycoprotein G is found to have oligosaccharides containing 5Man and 2GlcNAc residues, like the structure shown at the top of figure 1 (Tabas *et al.* 1978). Normal glycoprotein G has only complex oligosaccharide structures, indicating that Gn-T I-deficient cells are unable to make complex structures. Uninfected Gn-T I-deficient cells also accumulate the $(Man)_5(GlcNAc)_2$-Asn-X structure (Li & Kornfeld 1978).

It appears from the work with Gn-T I-deficient cells that the physiological substrate for Gn-T I is the $(Man)_5(GlcNAc)_2$-Asn-X intermediate (figure 1). However, Gn-T I can act on any one of the following three substrates (Harpaz & Schachter 1980*a*):



and

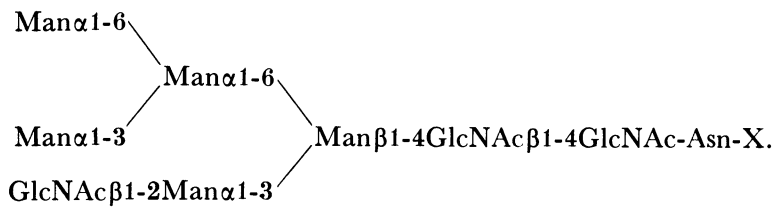



The action of Gn-T I on $(Man)_3(GlcNAc)_2$-Asn-X is the major physiological path in an interesting mouse lymphoma mutant that is unable to make dolichyl monophosphate mannose (Kornfeld *et al.* 1979; Chapman *et al.* 1980), and it may be the major synthetic path in normal cells under certain conditions (Rearick *et al.* 1981).

Johnston *et al.* (1966, 1973) first reported the presence in goat colostrum of a GlcNAc-transferase acting on $\alpha_1$-acid glycoprotein pretreated with neuraminidase, β-galactosidase and β-*N*-acetylglucosaminidase. A similar enzyme has been found in many tissues (Schachter & Roseman 1980) of the rat, pig, guinea-pig and human and has been found to be localized in the Golgi apparatus in rat liver. The high molecular mass acceptor detects both Gn-T I and II, and specific glycopeptide acceptors must be used to assay Gn-T I and II independently (Harpaz & Schachter 1980*a*). Gn-T I has been purified from bovine colostrum (Harpaz & Schachter 1980*a*), rabbit liver (Oppenheimer & Hill 1981) and pig liver and tracheal mucosa (Oppenheimer *et al.* 1981).

As is evident from figure 1, Gn-T I controls the entry of the synthetic pathway towards complex and hybrid structures. Several enzymes can act on the product of Gn-T I but not on the substrate, i.e. Gn-T I-dependent α-3/6-mannosidase(s), GDP-Fuc: β-*N*-acetylglucosaminide (Fuc to Asn-linked GlcNAc) α6-Fuc-transferase (α6-Fuc-T) and probably UDP-GlcNAc: glycopeptide β4-GlcNAc-transferase III (Gn-T III). Lack of action of these three enzymes explains, respectively, why there is no complex oligosaccharide synthesis, no fucose incorporation and no hybrid oligosaccharide synthesis until Gn-T I has acted. This is an example of a control point in which the addition of a single critical sugar residue allows previously impossible reactions to occur.

The product of Gn-T I activity is




Gn-T I appears to be specific for the terminal Manα1-3- residue of the core because it does not attack the other more peripheral terminal Manα1-3- residue of the substrate. Further, Gn-T I acts on the Manα1-3- terminus of $(Man)_3(GlcNAc)_2$-Asn-X and not on the equally available Manα1-6- terminus. The smallest effective substrate for Gn-T I is the trisaccharide Manα1-3Manβ1-4GlcNAc (Harpaz & Schachter 1980*a*).

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*Gn-T I-dependent α-3/6-mannosidase(s)*

The product of Gn-T I sits at a cross roads, i.e. it can be acted on by at least three (and probably four) different enzymes (figure 1). Although we have shown (Longmore & Schachter 1982) that α6-Fuc-T can act at this point to form a fucosylated non-bisected bi-antennary hybrid oligosaccharide, the natural occurrence of such a structure has not yet been demonstrated. Another possible pathway is directed towards the formation of hybrid structures via Gn-T III and Gn-T IV (this is discussed below). However, the most common fate for the product of Gn-T I (the 'main line' path) is the formation of complex oligosaccharides, containing 3Man residues, via the action of an unusual glycosidase that we have named Gn-T I-dependent α-3/6-mannosidase(s) (figure 1). This Golgi-localized enzyme or enzymes will act only on the product (but not on the substrate) of Gn-T I (Tabas & Kornfeld 1978; Harpaz & Schachter 1980*b*). The enzyme has been purified from rat liver Golgi-rich membranes (Tulsiani *et al.* 1981, 1982). Hen oviduct is rich in Gn-T III (Narasimhan *et al.* 1981*a*, *b*) but has relatively little Gn-T I-dependent α-3/6-mannosidase(s) activity. It is therefore not surprising to find that ovalbumin contains a large amount of bisected hybrid oligosaccharides (see below). Rat and pig liver Golgi-rich membranes have more Gn-T I-dependent α-3/6-mannosidase(s) activity than Gn-T III (unpublished data) activity and presumably make primarily complex oligosaccharides.

*UDP-GlcNAc : α-D-mannoside β2-GlcNAc-transferase II*

Lectin-resistant cells completely deficient in Gn-T I contain normal levels of UDP-GlcNAc:α-D-mannoside β2-GlcNAc-transferase II (Gn-T II), an enzyme that initiates the second branch or antenna on the Manα1-6- arm of the core (figure 1). Although Gn-T II has not yet been purified, it has been obtained free of Gn-T I from bovine colostrum (Harpaz & Schachter 1980*a*), pig liver and tracheal mucosa (Oppenheimer *et al.* 1981) and hen oviduct (unpublished data). The only effective substrate for Gn-T II is the product of Gn-T I-dependent α-3/6-mannosidase(s), i.e. glycopeptide MGn (figure 1). At least four enzymes can act on glycopeptide MGn, i.e. Gn-T II, Gn-T III, Gn-T IV and α6-Fuc-T (figure 1). Gn-T III and Gn-T IV, however, act relatively slowly on this substrate (table 1), and the formation of GnGn (with or without Fuc) via Gn-T II is no doubt the major fate of MGn. Gn-T II cannot act on Manα1-3(Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc-Asn-X, indicating that the prior action of Gn-T I is essential for its activity. Further, the presence of a bisecting GlcNAc prevents Gn-T II action (Harpaz & Schachter 1980*a*).

*UDP-GlcNAc : glycopeptide β4-GlcNAc-transferase III*

(*a*) *Hybrid oligosaccharide formation*

Some oligosaccharides are bisected by a GlcNAc residue linked β1-4 to the β-linked Man residue of the core. Tissues like hen oviduct, with a relative preponderance of Gn-T III over Gn-T I-dependent α-3/6-mannosidase(s), will insert a bisecting GlcNAc residue immediately after Gn-T I action (figure 1). This has a most interesting effect because the presence of a bisecting GlcNAc shuts off at least four different enzymes, i.e. Gn-T II, Gn-T IV, Gn-T I-dependent α-3/6-mannosidase(s) and α6-Fuc-T. The effect of Gn-T III action immediately after Gn-T I action is to 'freeze' oligosaccharide synthesis at the bisected bi-antennary hybrid stage. Synthesis stops except for elongation of the single antenna (initiated by Gn-T I) by




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addition of a Gal (and possibly a sialyl) residue. Sialylated hybrid structures have recently been found in lysosomal hydrolases.

Hen oviduct membranes are also rich in Gn-T IV (see below) and Gn-T IV may act on the product of Gn-T I before Gn-T III acts, thereby resulting in bisected tri-antennary hybrids.

Bovine rhodopsin (Liang *et al.* 1979) has been shown to contain glycopeptide MGn (figure 1) as well as the two non-bisected bi-antennary hybrid structures formed by the actions of Gn-T I and Gn-T I-dependent α-3/6-mannosidase(s) respectively (figure 1). The reason for the presence of these structures in rhodopsin is not known but interference with the actions of the mannosidase and Gn-T II is possible.

TABLE 1. GLYCOSYLTRANSFERASE SUBSTRATE SPECIFICITIES

(Hen oviduct membranes were used for studies with GlcNAc-transferase III (Gn-T III) at pH 5.7 and 7.0 (Narasimhan *et al.* 1981*a*, *b*), and GlcNAc-transferase IV (Gn-T IV) at pH 7.0 (Gleeson *et al.* 1982*a*, *b*). Golgi-enriched membranes from pig liver were used for the studies on GDP-Fuc:β-*N*-acetylglucosaminide (Fuc to Asn-linked GlcNAc) α1-6-fucosyltransferase (Fuc-T) (Longmore & Schachter 1982). These membranes are enriched 30-fold in Fuc-T relative to homogenate. One enzyme unit is 1 μmol $min^{-1}$.)

| | specific activity (μunits $mg^{-1}$) | | | |
|---|---|---|---|---|
| substrate† | Gn-T III, pH 5.7 | Gn-T III, pH 7.0 | Gn-T IV, pH 7.0 | Fuc-T, pH 8.0 |
| GnGn (+Fuc) | 85 | 73 | 83 | —‡ |
| GnGn (−Fuc) | — | 75 | 95 | 1800 |
| MGn (+Fuc) | ⩽13 | 10 | 18 | 0 |
| MGn (−Fuc) | — | — | — | 3100 |
| MM (+Fuc) | ⩽7 | ⩽16 | ⩽16 | 0 |
| MM (−Fuc) | — | — | — | 0 |
| GGn (+Fuc) | — | 48 | 30 | — |
| GnG (+Fuc) | — | ⩽5 | ⩽5 | — |
| GG (−Fuc) | — | ⩽8 | ⩽8 | 0§ |
| SS (−Fuc) | — | 0 | 0 | 0 |
| GnGn(Gn) (−Fuc) | — | — | ⩽3 | 0 |
| MGn(Gn) (−Fuc) | — | — | — | 0 |

† Structures of glycopeptides GnGn, MGn, GGn, GG and GnGn(Gn) are shown in figure 1. Glycopeptide MM is Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAc-Asn-X. GnG is similar to GGn except that the Gal residue is on the Manα1-3- arm instead of the Manα1-6- arm. SS is fully sialylated bi-antennary complex glycopeptide with both sialyl residues linked α2-6-. The glycopeptides are named according to the terminal sugar residues: M, Man; Gn, GlcNAc; G, Gal; S, sialyl; (Gn), bisecting GlcNAc.

‡ Not done, or not relevant.

§ Based on work with rat liver Golgi membranes that lack fucosyltransferase activities towards Galβ1-4GlcNAc-terminated acceptors (Munro *et al.* 1975).

### (*b*) *Bisected complex oligosaccharide formation*

Bisected complex oligosaccharides are relatively uncommon but have been found in several human multiple myeloma immunoglobulins (bisected bi-antennary complex oligosaccharides) and in ovotransferrin (bisected tri-antennary complex structures). Such structures cannot be formed by the action of Gn-T III at the 5-Man stage (figure 1), suggesting that there must be another point of entry for Gn-T III (Harpaz & Schachter 1980*b*). Gn-T III activity acting on glycopeptide GnGn (figure 1) has indeed been found in hen oviduct membranes (Narasimhan *et al.* 1981*a*, *b*). It is not known whether the Gn-T III activities acting at the 3-Man and 5-Man stages are one and the same enzyme.

The substrate specificity of Gn-T III is shown in table 1. It is evident that there is a relatively




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narrow 'window' of action (at glycopeptides MGn, GnGn and GGn; figure 1). As soon as both arms are galactosylated, Gn-T III action is no longer possible. As mentioned above, the introduction of a bisecting GlcNAc residue prevents further action of Gn-T IV. Thus if Gn-T III acts first, the synthetic pathway is 'frozen' into synthesis of bisected bi-antennary complex oligosaccharides (figure 1). If Gn-T IV acts before Gn-T III, synthesis of tri- and tetra-antennary oligosaccharides is possible. Glycopeptide GnGn is therefore at yet another important cross roads, leading into at least three different pathways, i.e. non-bisected bi-antennary complex, bisected bi-antennary complex, and tri- and/or tetra-antennary complex oligosaccharides; the last may remain unbisected or may become bisected via the action of Gn-T III.

### *UDP-GlcNAc : glycopeptide GnGn β4-GlcNAc-transferase IV*

Hen oviduct membranes are rich in UDP-GlcNAc:glycopeptide GnGn β4-GlcNAc-transferase IV (Gn-T IV), which initiates the third branch or antenna by the addition of a GlcNAc residue in β1-4- linkage to the Manα1-3- arm of glycopeptide GnGn (figure 1) (Gleeson *et al.* 1982*a*, *b*). The substrate specificity of Gn-T IV is shown in table 1. It shows the same narrow 'window' of specificity as Gn-T III. Thus highly branched (tri- and tetra-antennary) oligosaccharides, such as are believed to be present at increased levels in transformed cells, result presumably from a relative increase of Gn-T IV over both UDP-Gal:GlcNAc β4-galactosyltransferase (leading to bi-antennary complex structures) and Gn-T III (leading to bisected bi-antennary complex oligosaccharides). Prior action of Gn-T III prevents Gn-T IV action and directs the pathway into bisected bi-antennary complex structures. Galactosylation of both arms also prevents Gn-T IV action leading to bi-antennary complex oligosaccharides.

### *GDP-Fuc: β-N-acetylglucosaminide (Fuc to Asn-linked GlcNAc) α6-fucosyltransferase*

The enzyme that attaches Fuc in α1-6- linkage to the Asn-linked GlcNAc of the core can act at various points in the synthetic scheme (Longmore & Schachter 1982) (figure 1; table 1). The enzyme requires the presence of the GlcNAc incorporated by Gn-T I, but is prevented from acting by the incorporation of a bisecting GlcNAc by Gn-T III. Galactosylation of the antennae prevents α6-Fuc-T action.

## Biosynthesis of Ser(Thr)-GalNAc oligosaccharides

No attempt will be made here to cover this complex area (see Schachter & Williams (1982) for a recent review). We shall briefly mention three examples of control points in the *O*-glycosyl oligosaccharide synthetic pathways.

### *Ovine and porcine submaxillary mucins*

Ovine submaxillary mucin (o.s.m.) contains mainly GalNAc-Ser(Thr)-X and sialylα2-6GalNAc-Ser(Thr)-X oligosaccharides with small amounts (less than 2%) of Galβ1-3GalNAc-Ser(Thr)-X and fucosylated oligosaccharides. Porcine submaxillary mucin (p.s.m.) contains qualitatively similar structures but a much larger proportion of tetrasaccharides (and, in some pigs, pentasaccharides). The key control point (figure 2) occurs at GalNAc-Ser(Thr)-X, an intermediate that can be acted upon by two enzymes, UDP-Gal:GalNAc-mucin β1-3-Gal-




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transferase (mucin β3-Gal-T) and CMP-sialic acid:GalNAc-mucin α2-6-sialyltransferase (mucin α6-sialyl-T). Ovine submaxillary glands have a higher α6-sialyl-T/β3-Gal-T ratio than porcine glands, which leads to the sialyl-α2-6GalNAc- structure. The presence of the sialyl residue prevents β3-Gal-T action and 'freezes' the structure in the o.s.m. format. Conversely, in pig glands, β3-Gal-T functions more rapidly than α6-sialyl-T, which leads to the larger structures characteristic of p.s.m.

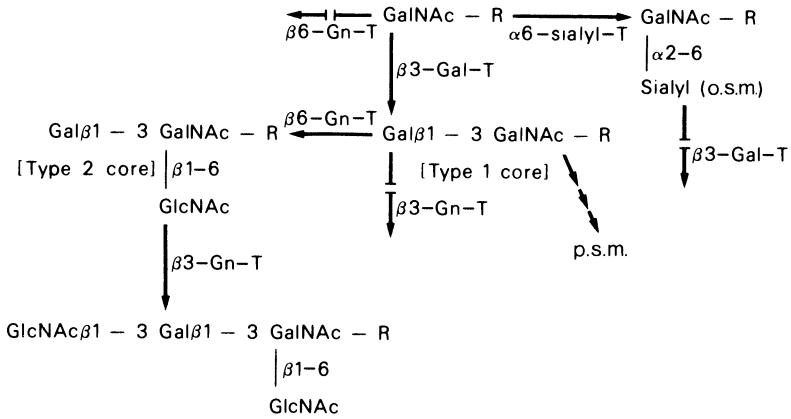


FIGURE 2. Paths involved in the synthesis of *O*-glycosyl oligosaccharides with type 1 and type 2 cores. Abbreviations: see text for the full names of the various transferases; o.s.m., ovine submaxillary mucin; p.s.m., porcine submaxillary mucin; R, Ser(Thr)-X.

### *Type 2 core synthesis and elongation*

The synthesis of type 2 core requires the action of UDP-GlcNAc:Galβ1-3GalNAc-mucin (GlcNAc to GalNAc) β1-6-GlcNAc-transferase (mucin β6-Gn-T) (Williams & Schachter 1980; Williams *et al.* 1980). Mucin β6-Gn-T does not act on GalNAc-mucin but requires the prior insertion of the Galβ1-3- residue by mucin β3-Gal-T(figure 2). Galβ1-3GalNAc-mucin is in fact a key intermediate in *O*-glycosyl oligosaccharide synthesis (Schachter & Williams 1982). The following enzymes compete for this intermediate in various tissues: (*a*) elongation of type 1 core by the addition of sialic acid (α2-3- to Gal or α2-6- to GalNAc), or of fucose (α1-2- to Gal), or of GlcNAc in various linkages to Gal, and (*b*) synthesis of type 2 core via mucin β6-Gn-T.

We have recently shown (unpublished data) the elongation *in vitro* of type 2 core by a novel enzyme present in porcine gastric mucosa and in other mucus-secreting tissues, i.e. UDP-GlcNAc:Galβ1-3(GlcNAcβ1-6)GalNAc-R β1-3-GlcNAc-transferase (mucin β3-Gn-T). This enzyme does not appear to act on Galβ1-3-GalNAc-R but requires the prior insertion of GlcNAcβ1-6- by mucin β6-Gn-T. The type 2 core structure Galβ1-3(GlcNAcβ1-6)GalNAc-R is also at a cross roads in *O*-glycosyl oligosaccharide synthesis (Schachter & Williams 1982) and can be elongated not only by the addition of GlcNAc in β1-3- linkage to Gal by β3-Gn-T but also by the addition of Gal or Fuc to the GlcNAc residue of the core.




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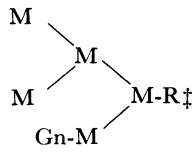
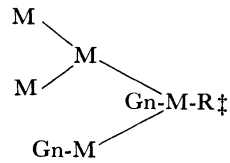
TABLE 2. CONTROL POINTS DURING OLIGOSACCHARIDE BIOSYNTHESIS

| type | substrate (figure 1 or 2) | key residue | enzymes active or inactive towards the substrate | synthetic fate† |
|---|---|---|---|---|
| | | | *active enzymes:* | |
| (*a*) competing enzymes | (i) product of Gn-T I:   | — | Gn-T I-dependent α3/6-mannosidase<br>Gn-T III<br>Gn-T IV<br>α6-Fuc-T | all com<br>bis/bi/hy<br>bis/tri/hy<br>— |
| | (ii) glycopeptide GnGn | — | Gal-T<br>Gn-T III<br>Gn-T IV<br>α6-Fuc-T | bi/com<br>bis/bi/com<br>tri/tetra/com (+/− bis)<br>— |
| | (iii) GalNAc-Ser(Thr)-X | — | α6-sialyl-T<br>β3-Gal-T | o.s.m.§<br>p.s.m.¶ |
| | | | *inactive enzymes:* | |
| (*b*) key residue 'freezes' pathway | (i)   | bisecting GlcNAc | Gn-T I-dependent α3/6-mannosidase<br>Gn-T IV<br>α6-Fuc-T | bis/bi/hy<br>bis/bi/hy<br>bis/bi/hy |
| | (ii) glycopeptide GnGn(Gn) | bisecting GlcNAc | Gn-T IV<br>α6-Fuc-T | bis/bi/com<br>bis/bi/com |
| | (iii) sialylα2-6-GalNAc-Ser(Thr)-X | sialyl residue | β3-Gal-T | o.s.m. |
| | | | *active enzymes:* | |
| (*c*) key residue 'channels' pathway | (i) product of Gn-T I (see above) | GlcNAc inserted by Gn-T I | same as (*a*) (i) above | same as (*a*) (i) above |
| | (ii) Galβ1-3-GalNAc-Ser(Thr)-X | Galβ1-3- | β6-GlcNAc-T | type 2 core synthesis |
| | (iii) Galβ1-3-(GlcNAcβ1-6-)GalNAc-Ser(Thr)-X | GlcNAcβ1-6- | β3-GlcNAc-T | elongation of type 2 core |

† Oligosaccharide products of the pathway. Abbreviations: bis, bisecting; bi, bi-antennary; tri, tri-antennary; tetra, tetra-antennary; com, complex; hy, hybrid.

‡ See figure 1 for full structures. M, Man; Gn, GlcNAc; R, GlcNAcβ1-4GlcNAc-Asn-X.

§ O.s.m., ovine submaxillary mucin.

¶ P.s.m., porcine submaxillary mucin.





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## Control points and key residues

Proteins and nucleic acids are essentially linear structures and are synthesized by a template mechanism. Oligosaccharides, being highly branched, cannot be assembled in this manner. Many factors undoubtedly play a role in oligosaccharide assembly, e.g. glycosyltransferase substrate specificity, the organization of the endomembrane assembly systems, the availability

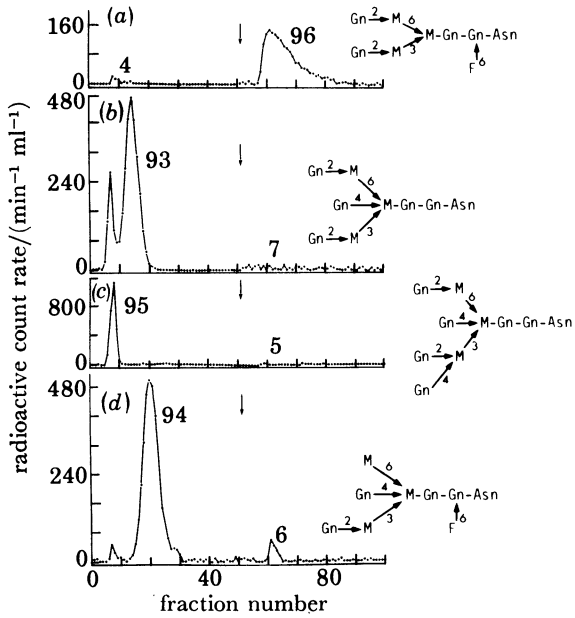


Figure 3. Radioactive glycopeptide standards were analysed on columns of Concanavalin A–Sepharose (0.7 cm × 15 cm) at pH 7.0. Arrows indicate start of elution with buffered 0.1 M methyl α-D-glucopyranoside. The figures near the peaks indicate the percentage radioactivity recovered in the peak relative to total radioactivity recovered from the column. Fraction size was 1.0 ml. Abbreviations in the structural formulae: M, Man; Gn, GlcNAc; F, Fuc. (*a*) *N*-[$^{14}$C]acetyl-glycopeptide GnGn. A radioactive count of 1940 min$^{-1}$ was loaded; recovery was 100 %. GnGn adheres firmly to the column and elutes in a typically broad peak with methyl α-D-glucopyranoside. (*b*) *N*-[$^{14}$C]acetyl-glycopeptide GnGn(Gn). Radioactive load, 3550 min$^{-1}$; recovery, 95 %. The introduction of a bisecting GlcNAc into GnGn to form GnGn(Gn) results in weakened binding to the lectin column. GnGn(Gn) comes through the column in retarded fashion (fractions 10–20). The material passing through totally unretarded (fractions 5–10) is a contaminant in the preparation (see Longmore & Schachter 1982). (*c*) *N*-[$^{14}$C]acetyl-glycopeptide GnGnGn(Gn). Radioactive load, 3060 min$^{-1}$; recovery, 98 %. Addition of a third antenna to GnGn(Gn) to form GnGnGn(Gn) results in a total lack of binding to the lectin column. This property allows the separation of the product of GlcNAc-transferase III (retarded) from the product of transferase IV (unretarded). (*d*) *N*-[$^{14}$C]acetyl-glycopeptide MGn(Gn); radioactive load, 3860 min$^{-1}$; recovery, 95 %. Glycopeptide MGn (see figure 1 for structure) binds tightly to Concanavalin A–Sepharose (data not shown). The introduction of a bisecting GlcNAc to MGn to form MGn(Gn) results in weakened binding, as indicated in the figure.

of substrates (acceptors and activated sugar donors), and the presence of cations and other cofactors. This discussion will deal with glycosyltransferase substrate specificity. A careful analysis of the synthetic paths discussed above indicates three types of control points: (*a*) two or more enzymes can compete for a common intermediate, (*b*) the addition of a 'key residue' to the growing oligosaccharide turns off further synthesis and 'freezes' the structure at a certain assembly stage, and (*c*) progression of the pathway is impossible unless a certain 'key residue'




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is inserted into the growing oligosaccharide. Table 2 summarizes examples of all three types of control points.

Control by competition seems to be exerted simply by the relative activities of various enzymes in a particular tissue. Thus hen oviduct is rich in Gn-T III and Gn-T IV relative to Gn-T I-dependent α-3/6-mannosidase(s), whereas in rat and pig liver exactly the reverse holds; the consequences have been discussed above. Similarly, ovine submaxillary glands have a higher α6-sialyl-T/β3-Gal-T ratio than porcine glands, leading to the structures typical of

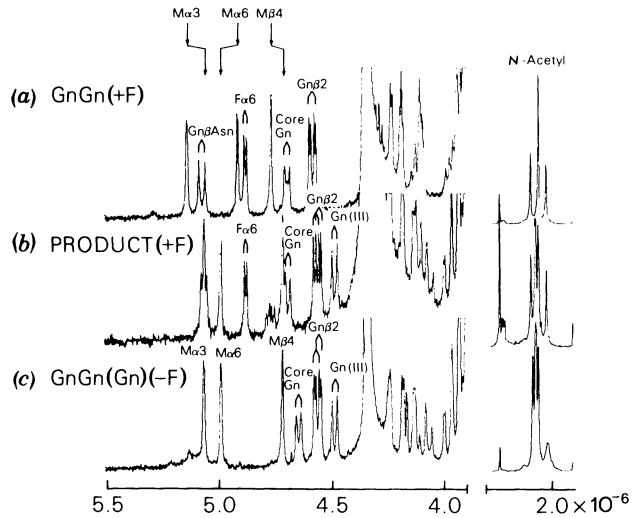


FIGURE 4. Anomeric and *N*-acetyl proton regions of the high resolution proton nuclear magnetic resonance (n.m.r.) spectra of glycopeptides. (*a*) Glycopeptide GnGn (with Fuc) prepared from human immunoglobulin G. (*b*) Glycopeptide GnGn(Gn), the product of GlcNAc-transferase III action on this GnGn preparation. (*c*) GnGn(Gn), without Fuc, isolated from hen ovalbumin. Glycopeptide nomenclature and structures are indicated in figure 1. The identification of the various signals is shown in the figure. Gn(III) indicates the signal for a bisecting GlcNAc residue. Note the dramatic shifts in the signals of all three Man residues caused by the presence of a bisecting GlcNAc residue. The signals for all four GlcNAc residues of GnGn and for Fuc are not appreciably altered by the introduction of a bisecting GlcNAc. The addition of a Fuc residue results in the appearance of its anomeric hydrogen signal (shown as Fα6) and in a downfield shift of the core GlcNAc (the residue attached to GlcNAc, not to Asn). It is curious that the signal for the Asn-linked GlcNAc (indicated by GnβAsn), hidden under the Manα1-3- signal, is not affected by attachment of a Fuc residue to the Asn-linked GlcNAc. The *N*-acetyl hydrogen resonances have been scaled down relative to the anomeric hydrogen signals. Addition of a bisecting GlcNAc residue results in an additional signal in the *N*-acetyl region.

o.s.m. and p.s.m. respectively. The other two types of control points depend on 'key residues' that either turn off or permit enzyme action. One key residue deserves particular attention because it acts at many points: the bisecting GlcNAc inserted by Gn-T III.

The presence of a bisecting GlcNAc turns off at least four enzymes (Gn-T II and IV, Gn-T I-dependent α-3/6-mannosidase(s), and α6-Fuc-T), suggesting a distortion in the three-dimensional structure of the oligosaccharide that prevents proper interaction between it and the various enzyme proteins. We have other evidence of interference in oligosaccharide–protein interaction by a bisecting GlcNAc residue. We have observed that the insertion of a bisecting GlcNAc into an oligosaccharide weakens its interaction with Concanavalin A–Sepharose (figure 3). This property has in fact proved useful in assaying Gn-T III since the substrate (glycopeptide GnGn, figure 3*a*) adheres firmly to the lectin column and requires 0.1 M methyl




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α-D-glucopyranoside for elution, whereas the product GnGn(Gn) adheres very weakly to the lectin (figure 3*b*).

The introduction of a bisecting GlcNAc also causes dramatic changes in the high-resolution proton nuclear magnetic resonance (n.m.r.) spectra of an oligosaccharide (Carver & Grey 1981). Figure 4(*a*, *b*, *c*) shows the anomeric hydrogen regions of the spectra of glycopeptides GnGn (with Fuc), GnGn(Gn) (with Fuc) and GnGn(Gn) (without Fuc) (see figure 1 for structures). The effect of a bisecting GlcNAc is seen by comparing figure 4*a* with figure 4*b*. Not only is there a dramatic shift in the signal for the β-linked Man (to which the bisecting GlcNAc is attached), but there are equally dramatic shifts in the signals for the other two Man residues. Further, the anomeric signal for the bisecting GlcNAc, labelled Gn(III) in figure 4, is in an anomalous position, i.e. it is further upfield than expected, in a position usually occupied by the anomeric hydrogen signal for Gal. This sort of result does not prove three-dimensional distortion by the bisecting GlcNAc but is certainly compatible with such a concept.

The three-dimensional structures of oligosaccharides and their interactions with proteins are relevant not only to the understanding of glycosyltransferase substrate specificity but also to the biological roles that complex carbohydrates play on the surfaces of cells and elsewhere. Our knowledge is currently very preliminary, but special n.m.r. techniques (e.g. the use of nuclear Overhauser enhancement) are currently being used by our group to study this problem.

This research was supported by the Medical Research Council of Canada and by N.I.H. grant no. HD-07889.

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