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Topical Review

Organization of Golgi Glycosyltransferases in Membranes: Complexity via Complexes

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Introduction

Much of the glycosylation of proteins, lipids, and proteoglycans of mammalian cells is catalyzed by glycosyltransferases located in the Golgi apparatus. This review will summarize recent data that delineates how those glycosyltransferases are organized in membranes during what can be called their "life cycle"; namely, the focus will be on the mechanisms responsible for and the molecules associated with the synthesis in the endoplasmic reticulum (ER), the targeting to and retention in the Golgi, and finally the turnover of individual glycosyltransferases. This review also will emphasize recent reports that illustrate exciting new features of the control of glycosylation resulting from the formation of complexes between and among Golgi enzymes.

Golgi Structure

Any consideration of the organization of Golgi resident enzymes must be made in the context of our current understanding of the mechanisms responsible for the formation and maintenance of the Golgi. For much of the last quarter of the twentieth century, the predominant model for protein transport through the Golgi was a vesicular transport-stable compartment model, but in the last several years a cisternal progression/maturation model has regained favor (Elsner, Hashimoto & Nilsson, 2003; van Vliet et al., 2003). In this latter model each new membrane arises by budding from the ER and coalescence into a vesicular tubular cluster (VTC). As a part of this budding process, newly synthesized proteins, including glycosyltransferases destined to become Golgi residents, are recruited as cargo into coat protein COPII-coated vesicles. As the nascent VTC moves forward, it matures to form sequentially the cis, medial, and trans Golgi cisternae and finally the trans Golgi network (TGN). This maturation is accomplished by the retrograde trafficking of COPI-coated vesicles. These vesicles are enriched at least three-fold in Golgi resident glycosylation enzymes as compared to anterograde cargo molecules by a process mediated by the small GTPase arf-1 (Lanoix et al., 1999). Clearly, this cisternal progression model will require future modification and already must be refined to take into account data showing forward transport of some COPI vesicles (Elsner, Hashimoto & Nilsson, 2003).

Glycosyltransferase Synthesis

Nearly all Golgi glycosyltransferases are type-II membrane proteins, meaning that they have an N-terminal cytoplasmic domain, a single-pass transmembrane domain (TMD), a so-called stem or stalk region, and finally a catalytic domain at the carboxy terminus that extends into the Golgi lumen. The life cycle of Golgi glycosyltransferases begins with the cotranslational translocation of the lumenal domain across the ER membrane. Many of these enzymes then become decorated with N-linked carbohydrate chains (Kleene & Berger, 1993) that aid in their proper folding via the calnexin-calreticulin chaperone system (Helenius et al., 1997). Interestingly, one of the glycosyltransferases that lack any consensus sites for N-glycosylation, core 1 β1,3GalT (Ju et al., 2002a), requires the unique chaperone Cosmc to achieve proper folding (Ju & Cummings, 2002). Cosmc and core 1 β1,3GalT form a physical complex, and in the absence of functional Cosmc, core 1 β1,3GalT is degraded in the proteosomes. Cosmc appears specific for core 1 β1,3GalT; whether there

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Table 1. Monomeric glycosyltransferases

Transferase	Reference(s)
Heparan sulfate N-deacetylase/N-sulfotransferase	Mandon et al., 1994
SpsA	Charnock and Davies, 1999
Polypeptide GalNAc transferases T1 and T2	Clausen and Bennett, 1996
α 1,3 GalT	Henion et al., 1994; Chen et al., 2000b
ST8Sia IV polysialyltransferase	Angata et al., 2001
Heparan sulfate 6-sulfotransferase	Habuchi, Habuchi, and Kimata, 1995

Table 2. Glycosyltransferase homodimers

Transferase	Reference(s)
β1,4GalT	Navaratnam et al., 1988; Yamaguchi and Fukuda, 1995
GM2 synthase	Li et al., 2000
α2,6-sialyltransferase	Ma and Colley, 1996; Qian, Chen, and Colley, 2001
GlcAT-I	Ouzzine et al., 2000
α1,3FucT VI	Borsig et al., 1998
core 2 β1,6 N-GlcNAcT	Yen et al., 2003
α1,2FucT	Opat, Houghton, and Gleeson, 2000
Core 1 β1,3 GalT	Ju, Cummings, and Canfield, 2002
Fringe	Correia et al., 2003
α3,4FucTIII	Sousa et al., 2003
GD3 synthase	Daniotti et al., 2000; Bieberich et al., 2000
EXT1, EXT2	McCormick et al., 2000
GlcNAcT-V	Sasai et al., 2001

are additional chaperones specific for other glycosyltransferases remains to be determined.

Some glycosyltransferases remain as monomeric proteins (Table 1), but several become homodimers in the ER that possess intersubunit disulfide bonds (Table 2). Data concerning the monomer-dimer status of glycosyltransferases is reviewed here because of the fundamental importance of oligomerization to Golgi targeting (*see* below). Intersubunit disulfides have been found in every possible domain: in the TMD domain of β1,4GalT, α3,4FucT-III, and α2,6-sialyltransferase; in the TMD or cytoplasmic domain of α1,3FucT-VI; in the stem of GlcAT-I; and in the stem and catalytic domains of GM2 synthase. An intersubunit disulfide was detected at Cys 235 within the catalytic domain of core 2 β1,6 N-GlcNAcT (Yen et al., 2003), but the authors questioned whether this disulfide might have formed during storage because the native enzyme from kidney was a monomer (Sekine et al., 1994). Finally, GlcNAcT-V forms homodimers and homo-oligomers through disulfides in the stem region and possibly also in the catalytic domain (Sasai et al., 2001).

The relationship between dimerization and enzyme activity varies for each protein. Generally those dimeric enzymes possessing intersubunit disulfides outside the catalytic domain are active in both the monomeric and dimeric forms. Both the monomers and dimers of core 1 β1,3GalT, for which the locations of the intersubunit disulfides are not known, are active as well (Ju, Cummings & Canfield, 2002b). GM2 synthase is unusual in that it requires dimerization for activity (Li et al., 2000). α2,6-Sialyltransferase (α2,6ST) forms an active monomer in the ER but roughly a third then forms an inactive disulfide-bonded homodimer (Ma & Colley, 1996) that requires Cys-24 in the TMD for dimerization (Qian, Chen & Colley, 2001).

Departure from the ER

At one time, occupancy of cargo molecules into anterograde vesicles that leave the ER was thought to be by default. However, it is now clear that cargo must be recruited via a Sar/Sec23-dependent mechanism. An exciting new finding is that exit from the ER of many Golgi glycosyltransferases is mediated by a dibasic motif within the cytoplasmic domain and near the transmembrane domain (Giraudo & Maccioni, 2003a). This dibasic motif interacts with the small GTPase Sar1 that in turn is involved in the recruitment of Sec23/24p to the ER membrane. One aspect of this system that initially appeared confusing was that this same dibasic motif also is responsible for ER retention of other type-II membrane proteins (Schutze, Peterson & Jackson, 1994). The difference in these signals is that the ER retention/retrieval signal

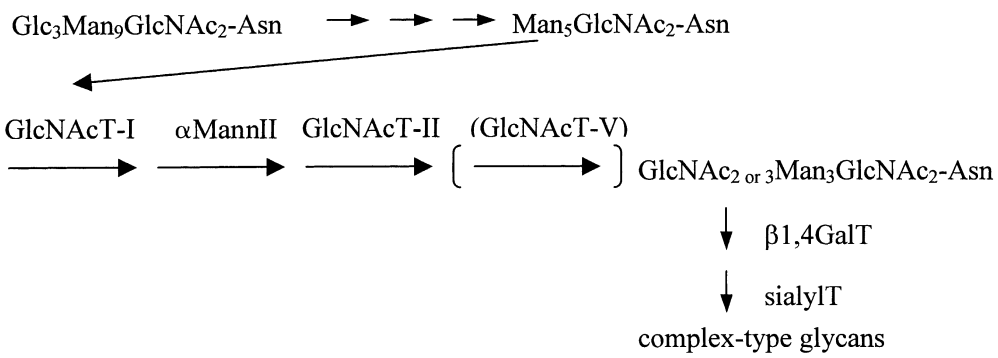

Fig. 1. N-linked glycosylation pathway in mammalian cells. Note that glycosylation by GlcNAcT-V is optional and requires the prior action of GlcNAcT-II. Complexes described in the text: the physical association of GlcNAcT-I and α-MannII (Nilsson et al., 1994); the presence of GlcNAcT-I and -II in high molecular weight, detergent insoluble complexes (Opat et al., 2000), whereas similar complexes produced at another pH contained α2,6ST (Chen et al., 2000a); and homo-oligomers of GlcNAcT-V (Sasai et al., 2001).

must be about 40 amino acids from the membrane, whereas the ER exit signal must be very near the membrane.

Glycosyltransferase Targeting to the Golgi: Glycosyltransferase Locations

Ever since the discovery of the remarkable morphology of the stacked Golgi cisternae and then the later realization that the Golgi was the site of major steps of glycosylation, there has been much interest in defining the sub-Golgi locations of glycosyltransferases. This interest was based partly on the functional consequences of compartmentalization; e.g., that glycosyltransferase locations could determine which of two or more biosynthetic pathways would be utilized and that co-localization of enzymes within a biosynthetic pathway could improve the efficiency of synthesis. Examples of each case are reviewed below. The locations of the glycosyltransferases of each major biosynthetic pathway are described next.

N-Linked Glycosylation

The landmark studies of the Kornfeld group (Kornfeld & Kornfeld, 1985) demonstrated that the enzymes responsible for synthesis and remodeling of the N-linked carbohydrate chains of glycoproteins were organized in the order in which they act, meaning that enzymes catalyzing earlier steps were located in compartments cis to late-acting enzymes (Fig. 1). Whereas initially this demarcation was thought to be strict, now it is clear that each enzyme is distributed in a gradient centered over a particular region of the Golgi. As a result of these gradients, there is overlap of enzyme distributions (Rabouille et al., 1995) and competition for substrates between enzymes of different pathways. Nevertheless, the locations of key enzymes of N-linked glycosylation remain gold standards for defining regions of the Golgi pathway; e.g., GlcNAcT-I for the medial Golgi and α2,6ST for the TGN.

Mucin-Type O-Glycosylation

Mucin-type O-linked glycosylation is initiated by the polypeptide:GalNAc transferase (ppGaNTase) family that may contain as many as 24 family members in mammals (Ten Hagen, Fritz & Tabak, 2003). Initial immuno-electronmicroscopic studies detected strongest ppGaNTase reactivity in the cis Golgi (Roth et al., 1994). Following the advent of cloning of ppGaNTase isoforms, epitope-tagged ppGaNTase T1 was found to be distributed throughout the Golgi stack, while T2 and T3 were more abundant in trans and medial cisternae than in cis cisternae (Rottger et al., 1998), suggesting that initiation of O-linked chains could occur throughout the Golgi. However, an obvious caveat of such localization by immunoreactivity is that the mere presence of an enzyme does not guarantee that the enzyme can function at that location (Varki, 1998). As a result, various investigators have employed domain-swapping experiments and other techniques to define the functional locations of glycosyltransferases (Sjoberg & Varki, 1993; Grabenhorst & Conradt, 1999).

Subsequent steps of O-linked glycosylation are carried out by many enzyme families, some of which glycosylate both N- and O-linked chains. Others add modifications such as sulfate. The sulfotransferase family, like the glycosyltransferases, is composed of Golgi resident type-II membrane proteins. Recently, Bertozzi's group found that the location of sulfotransferase (GlcNAc6ST) isoforms was critical to the biosynthesis of L-selectin ligands (De Graffenried & Bertozzi, 2003). They localized GlcNAc6ST isoform 1 in the TGN and isoform 3 in the cis Golgi, whereas

isoform 2 was present throughout the Golgi. Each GlcNAc6ST acts only on oligosaccharides having terminal GlcNAc and cannot utilize as substrates chains to which another terminal sugar has been added to the GlcNAc. The enzyme that adds the core 2 terminal GlcNAc, O-linked core 2 GlcNAcT-I, is located in the cis/medial Golgi. Therefore, because the sulfotransferases must act before another sugar is added to the terminal GlcNAc, the authors proposed that only GlcNAc6ST-2 or −3 could act on O-linked structures in the proximal Golgi to form the core sulfo-GlcNAc. In contrast, isoforms 1 or 2 can act in the TGN to catalyze the formation of N-linked sulfo-GlcNAc or sulfo-GlcNAc at the more peripheral locations on O-linked chains. This localization pattern illustrates a general principle seen in several other Golgi enzyme families such as the sialyltransferases, namely, that enzymes specific for O-linked glycosylation are located only in proximal compartments, whereas enzymes acting on both N- and O-linked chains are located throughout the Golgi.

Proteoglycan Glycosylation

As an example of proteoglycan biosynthesis, heparan sulfate biosynthesis begins with the addition of the first sugar, xylose, of the linkage region tetrasaccharide (GlcA-Gal-Gal-Xyl) to a Ser or Thr of the core protein (*see* Fig. 4), a process that occurs in the ER and perhaps in the ER-Golgi intermediate compartment as well. The tetrasaccharide is completed by the addition of two Gal and one GlcA, followed by chain polymerization by alternating addition of GlcNAc and GlcA by the enzymes EXT1 and EXT2. Final modification of the polymer is achieved by N-deacetylation/N-sulfation of GlcNAc by a bifunctional N-deacetylase/N-sulfotransferase (NDST1), epimerization of GlcA to iduronic acid (IdoA), and O-sulfation. All of the enzymes responsible for these steps from addition of the first Gal to 2-O-sulfation were colocalized with the medial Golgi marker αMannII in CHO cells (Pinhal et al., 2001). However, in mouse LTA cells NDST1 was located in the TGN (Humphries et al., 1997), and in rat liver sulfated polymer synthesis requires intercompartmental transport between Golgi cisternae (Fernández & Warren, 1998). These apparent contradictions probably reflect differences between the relatively simple Golgi of CHO cells and the Golgi of cells more heavily engaged in secretion.

Glycosphingolipid Glycosylation

Biosynthesis of glycosphingolipids (Maccioni, Giraudo & Daniotti, 2002) begins with the synthesis of ceramide at the cytosolic surface of the ER. Initial glycosylation to create GlcCer occurs at the cytosolic face of all Golgi cisternae (Jeckel et al., 1992). Because all subsequent glycosylation steps occur in the Golgi lumen, GlcCer must be translocated by an as yet unidentified translocase. Although there may be differences among cell types in terms of enzyme locations, generally LacCer synthase, GM3 synthase, and GD3 synthase are all distributed broadly across the proximal Golgi and the TGN as well. In contrast, GM2 synthase and enzymes that produce more complex gangliosides are restricted to the TGN.

In concluding this section on the locations of glycosyltransferases, two additional issues need to be mentioned for completeness. First, there are differences in the locations of glycosyltransferases between different cell types even within the same tissue (Roth et al., 1986). Second, in addition to the Golgi being the major location for glycosyltransferases, there are other locations where glycosyltransferases have been detected, such as the plasma membrane and axons (Berger, 2002). These "ectopic" locations may be most obvious in tissues or primary cell cultures as compared to cell lines. Both of these issues indicate that because much of our knowledge of glycosyltransferase organization has come from studies with cultured cell lines, we must remain open and alert to the possibility that unexpected differences may exist in tissues or different cell types.

Mechanisms for Golgi Targeting and Retention

Based partly on the characterization of specific peptide signals for various targeting events such as the KDEL signal for retention of soluble proteins in the ER (Munro & Pelham, 1987), much effort was spent during the 1990s, attempting to identify similar Golgi targeting and retention signals. Considering the lack of sequence homology between glycosyltransferases, it may not be surprising in retrospect that no specific targeting sequences have been identified. However, one general theme is that the TMD has a major effect on Golgi localization (Colley, 1997; van Vliet et al., 2003). Other regions of some glycosyltransferases were shown to contain targeting signals as well suggesting that these glycosyltransferases may possess multiple signals for Golgi targeting. However, with the realization that many of these enzymes are present in protein complexes (*see* below), it seems probable at least in some cases that the actual targeting signals may have resided in other members of the complexes rather than in the enzyme being studied at the time.

Partly because of the lack of identification of specific Golgi-targeting sequences, two models arose to explain the mechanisms responsible for targeting of glycosyltransferases to and their retention in the Golgi. The first was the lipid bilayer thickness or sorting model (Bretscher & Munro, 1993), which was

Table 3. Glycosyltransferase complexes

Transferase complex	Reference(s)
αMannII and GlcNAcT-I	Nilsson et al., 1994
Man pol-I and -II	Jungmann and Munro, 1998; Jungmann, Rayner, and Munro, 1999; Stolz and Munro, 2002
GlcNAcT-I and -II in high MW complex	Opat, Houghton, and Gleeson, 2000
α2,6ST in high MW complex	Chen et al., 2000a
GlcNAcTV homo-oligomers	Sasai et al., 2001
GM2 synthase and GM1 synthase	Giraudo, Daniotti, and Maccioni, 2001
LacCer synthase, GM3 synthase, GD3 synthase	Giraudo and Maccioni, 2003b
GM2 synthase and GD3 synthase	Bieberich et al., 2002
EXT1 and 2	McCormick et al., 2000
GlcA epimerase and the 2-O sulfotransferase	Pinhal et al., 2001

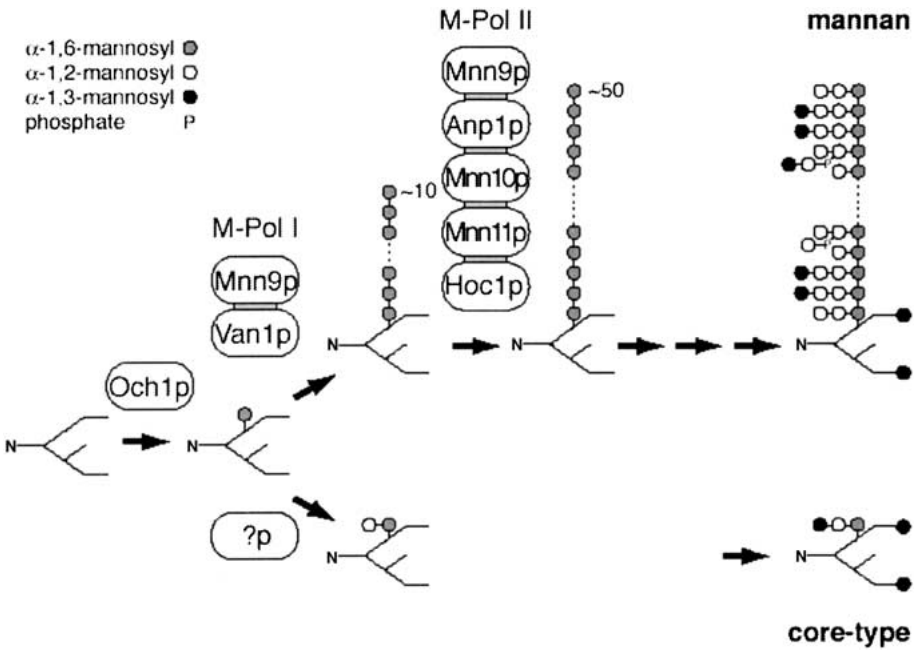

Fig. 2. Mannan formation in yeast. The complexes M-Pol I and II are described in the text. Reproduced, with permission, from Stolz, J., Munro, S. 2002. The components of the *Sacchoromyces cerevisiae* mannosyl transferase complex M-Pol I have distinct functions in mannan synthesis. *J. Biol. Chem.* **278:** 7624–7629.

based on the observations that: (1) the lipid bilayer increases in thickness due to increasing concentrations of cholesterol and sphingolipids from the ER through the Golgi to the plasma membrane; and (2) generally Golgi proteins have shorter transmembrane domains (TMD) than plasma membrane proteins. A variation on this latter theme that the physical characteristics of the TMD are important for Golgi targeting was the recent calculation that the overall hydrophobicity of the TMD and flanking regions of Golgi residents was lower than for proteins that occupy post-Golgi locations (Yuan & Teasdale, 2002). However, several recent observations indicate that the bilayer thickness model by itself cannot explain all aspects of Golgi targeting; one obvious example is the fact that some soluble forms of glycosyltransferases, which have lost their TMDs, are retained in the Golgi as a result of being associated in complexes (*see* below).

The second model for Golgi targeting and retention is the oligomerization or aggregation model that was originally devised by Machamer to explain the Golgi targeting of a viral protein (Machamer, 2001). The essence of this model is that the formation of homo- or hetero-oligomers of glycosyltransferases within Golgi cisternae would prevent their delivery to secretory vesicles and would therefore result in their Golgi retention. In fact, Roseman had predicted in 1970 the existence of such oligomers or complexes (Roseman, 1970). It is now clear that oligomerization plays a major role in Golgi targeting, as complexes have been identified in each major glycosylation category, namely, for glycoproteins, proteoglycans, and glycolipids, as described below. It is important to note here, however, that Golgi resident glycosyltransferases exhibit rapid diffusion within Golgi membranes of living cells, indicating that oligomerization does not lead to immobilization at

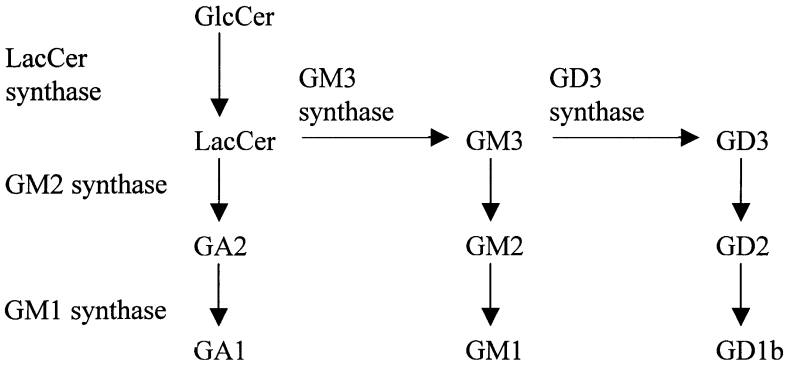

Fig. 3. Ganglioside biosynthetic pathway. Complexes described in the text: GM2 synthase and GM1 synthase (Giraudo et al., 2001); LacCer synthase, GM3 synthase and GD3 synthase (Giraudo & Maccioni, 2003b); and GM2 synthase and GD3 synthase (Bieberich et al., 2002). Note that GM2 synthase and GM1 synthase glycosylate the respective substrates in all three pathways shown vertically.

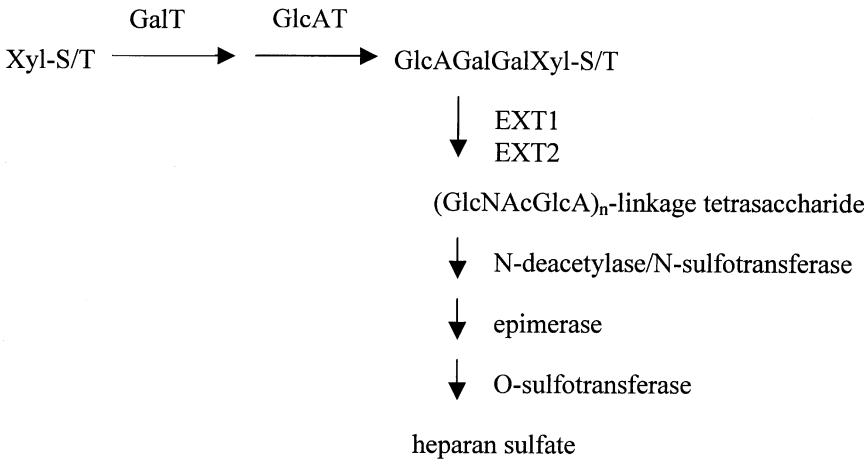

Fig. 4. Heparan sulfate biosynthetic pathway. Complexes described in the text: EXT1 and 2 (McCormick et al., 2000); and GlcA epimerase and 2-O sulfotransferase (Pinhal et al., 2001). S/T refers to Ser/Thr sites for O-glycosylation.

least of the enzymes studied to date (Cole et al., 1996).

Complexes or Oligomers in N-Linked Glycosylation

Warren's group documented the formation of complexes between Golgi enzymes involved in N-linked glycosylation using a novel approach. They reported that α mannosidase II (αMannII) and GlcNAcT-I (Fig. 1), two medial Golgi residents in HeLa cells, were physically associated (Table 3), whereas neither was associated with β1,4GalT, a distal Golgi resident (Nilsson et al., 1994). These complexes were found to be the result of interactions between charged residues in the lumenal domains (Nilsson et al., 1996). These results led to the "kin hypothesis", which proposed that enzymes located in the same compartment might form complexes (Nilsson et al., 1993). The clever approach used in this study of tagging one glycosyltransferase with an ER-targeting signal and then showing that this caused a second Golgi resident to also be retained in the ER has been used by others to demonstrate homodimer formation (Zhu et al., 1997) and heterooligomer formation (Pinhal et al., 2001). However, Munro, using this kin-recognition assay, did not detect complex formation between the distal Golgi residents α2,6ST and GalT1 (Munro, 1995), suggesting that this type of association may be specific for medial Golgi enzymes and not for those in the late Golgi.

Munro's group characterized two complexes, M-pol I and II (Fig. 2), formed by enzymes involved in the mannosylation of yeast N-linked chains of the cell-wall mannan (Jungmann & Munro, 1998; Jungmann, Rayner & Munro, 1999; Stolz & Munro, 2002). Both of these complexes occupy the cis Golgi. M-pol I initiates the formation of the α1,6-linked polymannose backbone and consists of a heterodimer of the proteins Mnn9p and Van1p, with a single copy of each present in the complex. Based on mutations in the conserved DXD motif of both components, Mnn9p appears to add the first α1,6-linked mannose and then Van1p is required for addition of subsequent mannoses perhaps in cooperation with Mnn9p. M-pol II is a huge complex of >1 million daltons, and its function is to extend the α1,6-linked polymannose backbone into a long polymer. This complex contains Mnn9p, as does M-pol I, plus four other components, Mnn10p, Anp1p, Mnn11p, and Hoc1p. Mnn10p and Mnn11p appear to be responsible for most of the α1,6 polymerizing activity. No

Table 4. List of cleaved and released glycosyltransferases

Enzyme	Cleavage site(s)	Reference(s)
α2,6ST Tyr form	YEALTA‖Q|A|K|EFQMPK[a]	Ma et al., 1997; Kitazume et al., 2003
β1,4GalT	GELR‖TGGARP[a]	Strous, 1986; Masri, Appert, and Fukuda, 1988; D'Agostaro, Bendiak, and Tropak, 1989
ppGaNTase T1	KKER‖GLPAGDV[a]	Homa et al., 1993
α1,3FucT-VI	*FFS*‖*Y*|*L*R|VSQDD[a]	Borsig et al., 1998; Grabenhorst and Conradt, 1999
α1,3GalT	QKDWW‖FPS|W[a]	Cho and Cummings, 1997; Cho, Yeh, and Cummings, 1997
GM2 synthase	*GLL*‖*YA*STRDA[a]	Jaskiewicz et al., 1996
EXT2	nd	Lind et al., 1998
EXTL2	nd	Kitagawa, Shimakawa, and Sugahara, 1999

[a] ‖ denotes the major cleavage site; |, a minor cleavage site(s); the TMD is underlined. The α2,6ST major cleavage site (Kitazume et al., 2003), the ppGaNTase T1 site, the α1,3GalT major cleavage site, and the β1,4GalT site (Masri, Appert & Fukuda, 1988) are 10, 12, 14, and 34 amino acids from the TMD, respectively; *nd*, not determined.

studies have been reported as yet to define the regions of each component that are responsible for complex formation.

Mention should be made in passing that yeast O-mannosyltransferases form protein complexes as well (Girrbach & Strahl, 2003). However, because these enzymes are ER residents and each contains multiple transmembrane domains unlike typical Golgi glycosyltransferases, they are not discussed in detail here.

The studies of Warren's and Munro's groups described above identified specific associations of individual glycosyltransferases with each other. In contrast Gleeson's and Colley's groups have used physico-chemical membrane isolation techniques to identify glycosyltransferases in fractions possessing certain characteristics. Therefore, it is important to recognize that this latter approach, while being informative about the physical state of an enzyme, can only report the presence of enzymes in such fractions and is uninformative about whether those enzymes are actually forming complexes with each other.

Gleeson's group found that in transfected CHO cells the medial enzymes GlcNAcT-I and GlcNAcT-II were part of high-molecular-weight complexes, whereas two late Golgi enzymes, beta4GalT and alpha2FucT, were not (Opat, Houghton & Gleeson, 2000). In this study, complexes were defined primarily by their insolubility in Triton X-100, a condition that coincidentally also defines cholesterol-rich raft complexes. However, extraction with saponin to remove cholesterol did not alter the distribution of GlcNAcT-I, indicating that the complexes being observed were independent of rafts. Inclusion of GlcNAcT-I into these high-molecular-weight complexes was dependent not on the cytoplasmic domain or TMD but rather on the lumenal domain. In fact, a soluble form of GlcNAcT-I lacking the TMD also was detained in such complexes prior to being secreted from cells. Finally, charged residues in the stem region of GlcNAcT-I, shown previously to be important for kin recognition (Nilsson et al., 1996), were not required for inclusion of GlcNAcT-I into these high-molecular-weight complexes, indicating that this type of high-molecular-weight aggregate was different from the complexes formed by kin recognition. Thus, the finding that GlcNAcT-I participates in two types of oligomers indicates that our understanding of glycosyltransferase complexes is in its infancy.

Colley's group then clarified the question of whether any late Golgi residents might be incorporated into oligomers by finding that α2,6ST was included in such oligomers in rat liver and transfected COS-1 cells but only at the acidic pH of the late Golgi, namely pH 5.8-6.3 (Chen et al., 2000a). These results indicated that the Golgi cisternae microenvironment was important for oligomer formation and explained why Gleeson's group had not seen inclusion in oligomers for late Golgi residents because their Triton X-100 extractions were performed at pH 6.5 (Opat et al., 2000). As described above, α2,6ST exists in both an active monomer and an inactive dimer form. Interestingly, the dimer form was found preferentially in the Triton X-100-insoluble pellet, suggesting that dimer formation might facilitate or even initiate the formation of higher-molecular-weight oligomers. α2,6ST also exists as two molecular forms, one with a tyrosine at position 123 of the lumenal domain that is readily cleaved and released from cultured cells, while the other form with a Cys at position 123 is not cleaved but instead adopts a more stable Golgi localization (Ma et al., 1997). Interestingly, the Cys form more readily formed insoluble oligomers than the Tyr form.

GlcNAcT-V is unique among the glycosyltransferase complexes studied to date because this enzyme forms disulfide-bonded homo-oligomers (Sasai et al., 2001). Its stem region, while not required for enzyme activity, not only confers Golgi retention but also is required for homo-oligomer formation. Therefore, at least one of the four Cys residues in the stem region must be responsible for homo-oligomer formation.

Interestingly, however, a portion of both wild-type GlcNAcT-V, as well as a mutant lacking the stem, was released from cells not as a dimer but in a monomeric form. The authors proposed that the portion of GlcNAcT-V that failed to oligomerize was then susceptible to cleavage and secretion. It would seem equally possible either that cleavage might occur before oligomerization or that the two processes might be alternative choices that could occur simultaneously.

Complexes in Glycosphingolipid Glycosylation

All of the above studies dealt with complex formation among enzymes of N-linked glycosylation. Maccioni's group used co-immunoprecipitation and fluorescence resonance energy transfer in transfected CHO cells to detect specific complex formation between two enzymes of ganglioside biosynthesis (Fig. 3), GM2 synthase and GM1 synthase (Giraudo, Daniotti & Maccioni, 2001). Recently, this group also found complex formation among LacCer synthase, GM3 synthase, and GD3 synthase (Giraudo & Maccioni, 2003b). In both cases, complex formation was mediated by the TMD and flanking regions of the enzymes involved. Each of these complexes could facilitate conversion of initial precursor to final product because each complex contains enzymes acting in sequence in biosynthetic pathways (Fig. 3). In apparent conflict with Maccioni's results, Yu's group detected complex formation in neuroblastoma cells between GD3 synthase and GM2 synthase (Bieberich et al., 2002). Such a complex could facilitate the synthesis of the "b series" gangliosides GD2, GD1b, and GT1b. However, it is entirely possible that neuronal cells that are enriched in complex gangliosides may have devised means for increasing the efficiency of synthesis of these gangliosides, whereas such mechanisms might not be present in the CHO cells used by Maccioni's group; untransfected CHO cells produce GM3 as the only major ganglioside (Young, Jr., Lutz & Blackburn, 1992; Rosales Fritz, Daniotti & Maccioni, 1997).

Complexes in Proteoglycan Glycosylation

The heparan sulfate biosynthetic pathway has provided two examples of specific complex formation (Fig. 4). First, EXT1 and EXT2 have attracted special interest because most cases of hereditary multiple exostoses have been attributed to mutations in either one of these genes. As mentioned above, EXT1 and EXT2 are responsible for heparan sulfate polymerization by alternating addition of GlcNAc and GlcA. Tufaro's group (McCormick et al., 2000) found that EXT1 resided in the ER when expressed without EXT2, but when the two proteins were coexpressed, they occupied the Golgi. Using co-immunoprecipitation, they demonstrated that EXT1 and EXT2 form homo- and hetero-oligomeric complexes. EXT2 has essentially no glycosyltransferase activity by itself, but the EXT1-EXT2 complex possesses greater glycosyltransferase activity than either component by itself. Second, Esko's group used a variety of approaches to detect a physical association between the GlcA epimerase and the 2-O sulfotransferase (Pinhal et al., 2001) and in the same report found no complex formation between the initial Gal transferase and the GlcA transferase acting to produce the core tetrasaccharide.

In summary, complex formation provides not only a mechanism for localization of glycosyltransferases to Golgi compartments but also a means for increasing the efficiency of the steps of glycosylation by physically linking two or more enzymes in a pathway. Such enzyme complexes can result in channeling of substrate into an assembly line or provide a means for synthesizing polymers, as in the cases of yeast mannan and heparan sulfate. An alternative mechanism for achieving increased efficiency is with enzymes that possess more than one function. In heparan sulfate biosynthesis the complex formed by EXT1 and EXT2 has both GlcNAc and GlcA transferase activities, neither of which can be attributed solely to either subunit alone (McCormick et al., 2000), and NDST1 possesses both N-deacetylase and N-sulfotransferase activities.

With regard to the location for the formation of glycosyltransferase oligomers, Gleeson's group has proposed that an oligomer residing in the medial Golgi might form in the ER and move forward by cisternal progression. In the case of oligomers containing α2,6ST that require the low pH of the late Golgi for their formation, monomers and/or dimers of α2,6ST may be transported by anterograde vesicles or by cisternal progression and form oligomers upon their arrival in the TGN (Opat, van Vliet & Gleeson, 2001).

Complexes between Enzymes of Different Pathways

Whereas all those complexes or oligomers described above have involved interactions between glycosyltransferases within a single pathway, only one study has reported a complex between enzymes in two different pathways. Simmons et al. used two-hybrid assays and in vitro binding assays to demonstrate a direct interaction between EXT proteins of the heparan sulfate biosynthetic pathway (Fig. 4) and ppGaNTase-T5, a member of the GalNAc transferase family that initiates mucin O-linked chain biosynthesis (Simmons et al., 1999). The fact that this was the first such complex described between enzymes of different pathways is due simply to the fact that in most previous studies the authors focused only on one pathway. Therefore, it is highly likely that similar multi-pathway complexes exist, waiting for future investigators to discover them.

Turnover or Cleavage

Turnover rates (Hare, 1990) have been calculated for only a few Golgi glycosyltransferases and range from a half time for GM2 synthase of 1.7 h in CHO cells (Jaskiewicz et al., 1996) and 6 h in neuroblastoma cells (Bieberich et al., 2000), to 3–4 h for the α2,6ST Tyr form in CHO and HeLa cells and 6 h in COS-1 cells (Ma et al., 1997), and to 30 h for GD3 synthase in neuroblastoma cells (Bieberich et al., 2000). The aspect of glycosyltransferase turnover described most fully is the proteolytic cleavage and release of a soluble fragment of the lumenal domain that leaves the cell via the constitutive secretory pathway. Generally the cleavage site is at or near the border between the TMD and the stem region (Table 4). The responsible protease was identified as a cathepsin D-like protease in the case of GM2 synthase (Jaskiewicz et al., 1996). In the case of α2,6ST the initial cleavage was accomplished by beta secretase followed by trimming by exopeptidase(s) (Kitazume et al., 2003).

Almost all Golgi glycosyltransferases are cleaved. Among the few enzymes that are not cleaved and released are the blood group H α1,2FucT (Larsen et al., 1990) and α-MannII (K. Moremen, personal communication). The subtleties of the cleavage process were hinted at by two reports. As described above, α2,6ST with a Tyr at position 123 of the lumenal domain was cleaved and released from cultured cells with the turnover times noted above, whereas substitution of a Cys at position 123 prevented the enzyme from being cleaved and released (Ma et al., 1997). This difference in susceptibility to proteolytic processing was apparently related to rather subtle differences in the subcellular locations of the two enzyme forms; both were localized in the distal Golgi but a portion of the Tyr form appeared at the cell surface. Similarly, fusion of the catalytic domain of FucT VI to various cytoplasmic, transmembrane, and stem regions of other glycosyltransferases resulted in six of eight chimeric proteins being cleaved and released (Grabenhorst & Conradt, 1999), although no obvious correlation could be made between the Golgi localization of the donor enzyme and susceptibility to release.

With regard to those membrane-bound Golgi glycosyltransferases that are cleaved and released as soluble forms, a persistent, still unanswered question is whether the soluble forms have specialized functions either intra- or extracellularly. It is possible that cleavage to release the lumenal domain is simply a means for protein turnover. Although it has generally been assumed that sugar nucleotides are not present in the extracellular fluid, in fact several examples of extracellular glycosyltransferases remodelling extracellular glycoconjugates have been described (reviewed by Cho et al., Cho, Yeh & Cummings, 1997). Within the cell, soluble glycosyltransferases might have access to substrates that are not accessible to the membrane-bound form and vice versa; i.e., it is possible that what is seen as the normal glycosylation pattern of a cell may actually be the sum of the glycosylation profiles provided by membrane-bound enzymes plus additional glycosylation contributed by soluble enzymes during their passage through the secretory pathway.

Several reports have compared the activities in intact cells of soluble glycosyltransferases versus their full-length counterparts. A soluble form of α1,3-GalT was as efficient as its membrane- bound counterpart in galactosylating newly synthesized proteins in intact cells (Cho & Cummings, 1997). Similarly, soluble and membrane-bound GlcNAcT-I were comparable in their ability to glycosylate newly synthesized proteins in vivo (Opat et al., 2000). The authors attributed the activity of the soluble GlcNAcT-I to the inclusion of this soluble form in high-molecular-weight oligomers (*see* above). In striking contrast to α1,3-GalT and GlcNAcT-I, a soluble form of GM2 synthase was much less efficient in intact cells as compared to membrane-bound GM2 synthase in producing its product, GM2 ganglioside, despite the fact that both forms were fully functional in in vitro assays (Zhu et al., 1998). Similarly, although soluble α2,6 ST can sialylate cell surface glycoconjugates (Donadio et al., 2003), the efficiency of this soluble form in intact cells was much less than its membrane-bound counterpart even though both forms were active in vitro (Cho & Cummings, 1997). Finally, soluble forms of α2,6 ST, α1,3 FucTIII and α1,3 FucTVI were unable to glycosylate secretory glycoproteins coexpressed in doubly transfected cells (Grabenhorst, Costa & Conradt, 1997).

Summary and Future Studies

More Complexes/Oligomers?

Considering the fact that there are dozens of Golgi glycosyltransferases in eukaryotic cells, clearly the few enzyme complexes and oligomers reviewed here provide us with just an initial glimpse of the membrane organization of these enzymes. It is not surprising that the majority of glycosyltransferase complexes and oligomers that have been found to date are in the N-linked glycosylation pathway. Unlike mucin-type O-linked glycosylation that lacks a well-defined glycosylation consensus sequence or specific inhibitors of glycosylation, the N-linked glycosylation consensus sequence is fully described and the specific inhibitor tunicamycin has been in use for many years. Therefore, our knowledge of the N-linked pathway is years ahead of a similar understanding of O-linked glycosylation. Future investigation for complex formation between or among the

members of the ppGaNTase family that initiates mucin glycosylation may prove fruitful. Isoforms 7 and 10 prefer glycopeptide substrates, meaning a peptide region to which one GalNAc has already been added, whereas most other isoforms will glycosylate peptides lacking any glycosylation (Ten Hagen et al., 2003). Therefore, a complex formed between a peptide-specific isoform and a glycopeptide-specific isoform could increase the efficiency of mucin chain synthesis.

The glycosphingolipids (GSL) encompass dozens of exotic sugar structures that are grouped into globo-, lacto-, ganglio-, muco-, and gal- series. Except for the ganglioside pathway complexes described above, no complexes have been identified in the other pathways of GSL synthesis despite the fact that such a complex was proposed in the globo- pathway in 1980 (Kijimoto-Ochiai, Yokosawa & Makita, 1980). Because of the importance of the sphingolipids in cell signalling pathways (Hakomori, 2002), understanding the organization of GSL glycosyltransferases should continue to be a fruitful area in coming years.

Numerous other questions about glycosyltransferase organization await investigation. For those enzymes that are proteolytically cleaved such that the lumenal domain is released as a soluble form, what is the fate of the TMD and flanking region stub that remains in the Golgi membrane? In several cases that stub region has been shown to mediate complex formation. Therefore, does the stub compete with full-length copies of the same enzyme to block the formation of new complexes?

Are there specific chaperones for individual glycosyltransferases similar to Cosmc (Ju & Cummings, 2002) remaining be found? What is the relationship between the organization of sugar-nucleotide transporters and glycosyltransferases? What is the effect of substrates on the formation and stability of glycosyltransferase complexes and oligomers? Finally, the majority of studies on glycosyltransferase oligomerization have been performed with common cell lines like CHO cells. What new features of glycosyltransferase organization will become evident when tissues and more exotic cell sources are utilized?

Relationship between Glycosyltransferase Oligomers and Golgi Structure

When the idea of glycosyltransferase complexes or oligomers was originally conceived and the initial complexes were identified, the vesicular transport-stable compartment model for Golgi construction was dominant. Consistent with that model was the suggestion that the complexes or oligomers would be too large to be incorporated into the vesicles carrying cargo in an anterograde direction and, therefore, would be retained in the Golgi. Currently there is much evidence supporting the alternative cisternal progression/maturation model. How do such complexes or oligomers fit with that model? In this latter model Golgi resident proteins are not retained in specific cisternae but instead are transported via COPI coated vesicles in a retrograde direction. As a result the cisternae and their cargo progress forward while the resident Golgi proteins including the glycosyltransferases can maintain their steady-state cisternal localization. If this scenario is correct, then many questions await investigation. Which glycosyltransferases are incorporated into COPI vesicles; e.g., is there preferential incorporation of cis Golgi enzymes as compared to those in the late Golgi? What is the fate of glycosyltransferase complexes and oligomers? Are they too large to be incorporated into COPI vesicles? Are there processes occurring within the cisternae that control oligomerization and perhaps simultaneously regulate movement of those enzymes into COPI vesicles?

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References

Angata, K., Yen, T.Y., El Battari, A., Macher, B.A., Fukuda, M. 2001. Unique disulfide bond structures found in ST8Sia IV polysialyltransferase are required for its activity. *J. Biol. Chem.* **276:**15369–15377

Berger, E.G. 2002. Ectopic localizations of Golgi glycosyltransferases. *Glycobiology* **12:**29R–36R

Bieberich, E., MacKinnon, S., Silva, J., Li, D.D., Tencomnao, T., Irwin, L., Kapitonov, D., Yu, R.K. 2002. Regulation of ganglioside biosynthesis by enzyme complex formation of glycosyltransferases. *Biochemistry* **41:**11479–11487

Bieberich, E., Tencomnao, T., Kapitonov, D., Yu, R.K. 2000. Effect of N-glycosylation on turnover and subcellular distribution of N-acetylgalactosaminyltransferase I and sialyltransferase II in neuroblastoma cells. *J. Neurochem.* **74:**2359–2364

Borsig, L., Katopodis, A.G., Bowen, B.R., Berger, E.G. 1998. Trafficking and localization studies of recombinant α1,3-fucosyltransferase VI stably expressed in CHO cells. *Glycobiology* **8:**259–268

Bretscher, M.S., Munro, S. 1993. Cholesterol and the Golgi apparatus. *Science* **261:**1280–1281

Charnock, S.J., Davies, G.J. 1999. Structure of the nucleotide-diphospho-sugar transferase, SpsA from *Bacillus subtilis*, in native and nucleotide-complexed forms. *Biochemistry* **38:**6380–6385

Chen, C., Ma, J.Y., Lazic, A., Backovic, M., Colley, K.J. 2000a. Formation of insoluble oligomers correlates with ST6Gal I stable localization in the Golgi. *J. Biol. Chem.* **275:**13819–13826

Chen, X., Liu, Z., Wang, J., Fang, J., Fan, H., Wang, P.G. 2000b. Changing the donor cofactor of bovine alpha 1,3-galactosyltransferase by fusion with UDP-galactose 4-epimerase: More efficient biocatalysis for synthesis of alpha-Gal epitopes. *J. Biol. Chem.* **275:**31594–31600

Cho, S.K., Cummings, R.D. 1997. A soluble form of alpha1,3-galactosyltransferase functions within cells to galactosylate glycoproteins. *J. Biol. Chem.* **272:**13622–13628

Cho, S.K., Yeh, J.C., Cummings, R.D. 1997. Secretion of α1,3-galactosyltransferase by cultured cells and presence of enzyme in animal sera. *Glycoconjugate J.* **14:**809–819

Clausen, H., Bennett, E.P. 1996. A family of UDP-GalNAc: Polypeptide *N*-acetylgalactosaminyl-transferases control the initiation of mucin-type O-linked glycosylation. *Glycobiology* **6:**635–646

Cole, N.B., Smith, C.L., Sciaky, N., Terasaki, M., Edidin, M., Lippincott-Schwartz, J. 1996. Diffusional mobility of Golgi proteins in membranes of living cells. *Science* **273:**797–801

Colley, K.J. 1997. Golgi localization of glycosyltransferases: More questions than answers. *Glycobiology* **7:**1–13

Correia, T., Papayannopoulos, V., Panin, V., Woronoff, P., Jiang, J., Vogt, T.F., Irvine, K.D. 2003. Molecular genetic analysis of the glycosyltransferase Fringe in Drosophila. *Proc. Natl. Acad. Sci. USA* **100:**6404–6409

D'Agostaro, G., Bendiak, B., Tropak, M. 1989. Cloning of cDNA encoding the membrane-bound form of bovine beta 1,4-galactosyltransferase. *Eur. J. Biochem.* **183:**211–217

Daniotti, J.L., Martina, J.A., Giraudo, C.G., Zurita, A.R., Maccioni, H.J.F. 2000. GM3 alpha 2,8-sialyltransferase (GD3 synthase): Protein characterization and sub-Golgi location in CHO-K1 cells. *J. Neurochem.* **74:**1711–1720

De Graffenried, C.L., Bertozzi, C.R. 2003. Golgi localization of carbohydrate sulfotransferases is a determinant of L-selectin ligand biosynthesis. *J. Biol. Chem.* **278:**40282–40295

Donadio, S., Dubois, C., Fichant, G., Roybon, L., Guillemot, J.C., Breton, C., Ronin, C. 2003. Recognition of cell surface acceptors by two human alpha-2,6-sialyltransferases produced in CHO cells. *Biochimie* **85:**311–321

Elsner, M., Hashimoto, H., Nilsson, T. 2003. Cisternal maturation and vesicle transport: join the band wagon! *Mol. Membr. Biol.* **20:**221–229

Fernández, C.J., Warren, G. 1998. *In vitro* synthesis of sulfated glycosaminoglycans coupled to inter-compartmental Golgi transport. *J. Biol Chem.* **273:**19030–19039

Giraudo, C.G., Daniotti, J.L., Maccioni, H.J.F. 2001. Physical and functional association of glycolipid N-acetyl-galactosaminyl and galactosyl transferases in the Golgi apparatus. *Proc. Natl. Acad. Sci. USA* **98:**1625–1630

Giraudo, C.G., Maccioni, H.J.F. 2003a. ER export of glycosyltransferases depends on interaction of a cytoplasmic di-basic motif with Sar1. *Mol. Biol. Cell* **14:**3753–3766

Giraudo, C.G., Maccioni, H.J.F. 2003b. Ganglioside glycosyltransferases organize in distinct multienzyme complexes in CHO-K1 cells. *J. Biol. Chem.* **278:**40262–40271

Girrbach, V., Strahl, S. 2003. Members of the evolutionarily conserved PMT family of protein O-mannosyltransferases form distinct protein complexes among themselves. *J. Biol. Chem.* **278:**12554–12562

Grabenhorst, E., Conradt, H.S. 1999. The cytoplasmic, transmembrane, and stem regions of glycosyltransferases specify their in vivo functional sublocalization and stability in the Golgi. *J. Biol. Chem.* **274:**36107–36116

Grabenhorst, E., Costa, J., Conradt, H.S. 1997. Construction of novel BHK-21 cell lines coexpressing Golgi resident or soluble forms of human alpha 2,6-sialyltransferase and alpha 1,3/4-fucosyltransferases together with secretory glycoproteins. In: Carrondo, M.J.T., Griffiths, B., Moreira, J.L.P. (editors) From Vaccines to Genetic Medicine. Kluwer Academic Publishers, Dordrecht, The Netherlands, 481–487

Habuchi, H., Habuchi, O., Kimata, K. 1995. Purification and characterization of heparan sulfate 6-sulfotransferase from the culture medium of Chinese hamster ovary cells. *J. Biol. Chem.* **270:**4172–4179

Hakomori, S. 2002. The glycosynapse. *Proc. Natl Acad. Sci. USA* **99:**225–232

Hare, J.F. 1990. Mechanisms of membrane protein turnover. *Biochim. Biophys. Acta* **1031:**71–90

Helenius, A., Trombetta, E.S., Hebert, D.N., Simons, J.F. 1997. Calnexin, calreticulin and the folding of glycoproteins. *Trends Cell Biol.* **7:**193–200

Henion, T.R., Macher, B.A., Anaraki, F., Galili, U. 1994. Defining the minimal size of catalytically active primate α1,3 galactosyltransferase: Structure-function studies on the recombinant truncated enzyme. *Glycobiology* **4:**193–202

Homa, F.L., Hollander, T., Lehman, D.J., Thomsen, D.R., Elhammer, A.P. 1993. Isolation and expression of a cDNA clone encoding a bovine UDP-GalNAc:polypeptide N-Acetylgalactosaminyltransferase. *J. Biol. Chem.* **268:**12609–12616

Humphries, D.E., Sullivan, B.M., Aleixo, M.D., Stow, J.L. 1997. Localization of human heparan glucosaminyl N-deacetylase/N-sulphotransferase to the trans-Golgi network. *Biochem. J.* **325:**351–357

Jaskiewicz, E., Zhu, G., Bassi, R., Darling, D.S., Young, W.W. Jr. 1996. beta-1,4 N-acetylgalactosaminyltransferase (GM2 synthase) is released from Golgi membranes as a neuraminidase sensitive, disulfide-bonded dimer by a cathepsin D-like protease. *J. Biol. Chem.* **271:**26395–26403

Jeckel, D., Karrenbauer, A., Burger, K.N.J., Van Meer, G., Wieland, F. 1992. Glucosylceramide is synthesized at the cytosolic surface of various Golgi subtractions. *J. Cell. Biol.* **117:**259–267

Ju, T., Brewer, K., D'Souza, A., Cummings, R.D., Canfield, W.M. 2002a. Cloning and expression of human core 1 beta1,3-galactosyltransferase. *J. Biol. Chem.* **277:**178–186

Ju, T., Cummings, R.D. 2002. A unique molecular chaperone Cosmc required for activity of the mammalian core 1 beta 3-galactosyltransferase. *Proc. Natl. Acad. Sci. USA* **99:**16613–16618

Ju, T., Cummings, R.D., Canfield, W.M. 2002b. Purification, characterization, and subunit structure of rat core 1 Beta1,3-galactosyltransferase. *J. Biol. Chem.* **277:**169–177

Jungmann, J., Munro, S. 1998. Multi-protein complexes in the *cis* Golgi of *Saccharomyces cerevisiae* with α-1,6-mannosyltransferase activity. *EMBO J.* **17:**423–434

Jungmann, J., Rayner, J.C., Munro, S. 1999. The *Saccharomyces cerevisiae* protein Mnn10p/Bed1p is a subunit of a Golgi mannosyltransferase complex. *J. Biol. Chem.* **274:**6579–6585

Kijimoto-Ochiai, S., Yokosawa, N., Makita, A. 1980. Mechanism for the biosynthesis of Forssman glycolipid from trihexosylceramide. *J. Biol. Chem.* **255:**9037–9040

Kitagawa, H., Shimakawa, H., Sugahara, K. 1999. The tumor suppressor EXT-like gene EXTL2 encodes an alpha1,4-N-acetylhexosaminyltransferase that transfers N-acetylgalactosamine and N-acetylglucosamine to the common glycosaminoglycan-protein linkage region. The key enzyme for the chain initiation of heparan sulfate. *J. Biol. Chem.* **274:**13933–13937

Kitazume, S., Tachida, Y., Oka, R., Kotani, N., Ogawa, K., Suzuki, M., Dohmae, N., Takio, K., Saido, T.C., Hashimoto, Y. 2003. Characterization of alpha 2,6-sialyltransferase cleavage by Alzheimer's beta-secretase (BACE1). *J. Biol. Chem.* **278:**14865–14871

Kleene, R., Berger, E.G. 1993. The molecular and cell biology of glycosyltransferases. *Biochim. Biophys. Acta Rev. Biomembr.* **1154:**283–325

Kornfeld, S., Kornfeld, R. 1985. Assembly of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* **54:**631–664

Lanoix, J., Ouwendijk, J., Lin, C.C., Stark, A., Love, H.D., Ostermann, J., Nilsson, T. 1999. GTP hydrolysis by arf-1 mediates sorting and concentration of Golgi resident enzymes into functional COPI vesicles. *EMBO J.* **18:**4935–4948

Larsen, R.D., Ernst, L.K., Nair, R.P., Lowe, J.B. 1990. Molecular cloning, sequence, and expression of a human GDP-L-fucose:beta-D-galactoside 2-alpha-L-fucosyltransferase cDNA that can form the H blood group antigen. *Proc. Natl. Acad. Sci. USA* **87:**6674–6678

Li, J., Yen, T.-Y., Allende, M.L., Joshi, R.K., Cai, J., Pierce, W.M., Jaskiewicz, E., Darling, D.S., Macher, B.A., Young, W.W. Jr. 2000. Disulfide bonds of GM2 synthase homodimers: Antiparallel orientation of the catalytic domains. *J. Biol. Chem.* **275:**41476–41486

Lind, T., Tufaro, F., McCormick, C., Lindahl, U., Lidholt, K. 1998. The putative tumor suppressors EXT1 and EXT2 are glycosyltransferases required for the biosynthesis of heparan sulfate. *J. Biol. Chem.* **273:**26265–26268

Ma, J., Qian, R., Rausa, F.M., III., Colley, K.J. 1997. Two naturally occurring α2,6-sialyltransferase forms with a single amino acid change in the catalytic domain differ in their catalytic activity and proteolytic processing. *J. Biol. Chem.* **272:**672–679

Ma, J.Y., Colley, K.J. 1996. A disulfide-bonded dimer of the Golgi β-galactoside α2,6-sialyltransferase is catalytically inactive yet still retains the ability to bind galactose. *J. Biol. Chem.* **271:** 7758–7766

Maccioni, H.J., Giraudo, C.G., Daniotti, J.L. 2002. Understanding the stepwise synthesis of glycolipids. *Neurochem. Res.* **27:**629–636

Machamer, C.E. 2001. Golgi retention signals: Do membranes hold the key? *Trends Cell Biol.* **1:**141–144

Mandon, E.C., Kempner, E.S., Ishihara, M., Hirschberg, C.B. 1994. A monomeric protein in the Golgi membrane catalyzes both *N*-deacetylation and *N*-sulfation of heparan sulfate. *J. Biol. Chem.* **269:**11729–11733

Masri, K.A., Appert, H.E., Fukuda, M.N. 1988. Identification of the full-length coding sequence for human galactosyltransferase (beta-n-acetyglucosaminide: Beta1, 4-galactosyltransferase). *Biochem. Biophys. Res. Commun.* **157:**657–663

McCormick, C., Duncan, G., Goutsos, K.T., Tufaro, F. 2000. The putative tumor suppressors EXT1 and EXT2 form a stable complex that accumulates in the Golgi apparatus and catalyzes the synthesis of heparan sulfate. *Proc. Natl. Acad. Sci. USA* **97:**668–673

Munro, S. 1995. An investigation of the role of transmembrane domains in Golgi protein retention. *EMBO J.* **19:**4695–4704

Munro, S., Pelham, H.R.B. 1987. A C-terminal signal prevents secretion of luminal ER proteins. *Cell* **48:**899–907

Navaratnam, N., Ward, S., Fisher, C., Kuhn, N.J., Keen, J.N., Findlay, J.B.C. 1988. Purification, properties and cation activation of galactosyltransferase from lactating-rat mammary Golgi membranes. *Eur. J. Biochem.* **171:**623–629

Nilsson, T., Hoe, M.H., Slusarewicz, P., Rabouille, C., Watson, R., Hunte, F., Watzele, G., Berger, E.G., Warren, G. 1994. Kin recognition between *medial* Golgi enzymes in HeLa cells. *EMBO J.* **13:**562–574

Nilsson, T., Rabouille, C., Hui, N., Watson, R., Warren, G. 1996. The role of the membrane-spanning domain and stalk region of N-acetylglucosaminyltransferase I in retention, kin recognition and structural maintenance of the Golgi apparatus in HeLa cells. *J. Cell Sci.* **109:**1975–1989

Nilsson, T., Slusarewicz, P., Hoe, M.H., Warren, G. 1993. Kin recognition: A model for the retention of Golgi enzymes. *FEBS Lett.* **330:**1–4

Opat, A.S., Houghton, F., Gleeson, P.A. 2000. Medial Golgi but not late Golgi glycosyltransferases exist as high molecular weight complexes - Role of luminal domain in complex formation and localization. *J. Biol. Chem.* **275:**11836–11845

Opat, A.S., van Vliet, C., Gleeson, P.A. 2001. Trafficking and localisation of resident Golgi glycosylation enzymes. *Biochimie* **83:**763–773

Ouzzine, M., Gulberti, S., Netter, P., Magdalou, J., Fournel-Gigleux, S. 2000. Structure-function of the human Gal beta 1,3-glucuronosyltransferase (GlcAT-I). Dimerization and functional activity are mediated by two crucial cysteine residues. *J. Biol. Chem.* **275:**28254–28260

Pinhal, M.A.S., Smith, B., Olson, S., Aikawa, J., Kimata, K., Esko, J.D. 2001. Enzyme interactions in heparan sulfate biosynthesis: Uronosyl 5-epimerase and 2-O-sulfotransferase interact in vivo. *Proc. Natl. Acad. Sci. USA* **98:**12984–12989

Qian, R., Chen, C., Colley, K.J. 2001. Location and mechanism of alpha 2,6-sialyltransferase dimer formation. Role of cysteine residues in enzyme dimerization, localization, activity, and processing. *J. Biol. Chem.* **216:**28641–28649

Rabouille, C., Hui, N., Hunte, F., Kieckbusch, R., Berger, E.G., Warren, G., Nilsson, T. 1995. Mapping the distribution of Golgi enzymes involved in the construction of complex oligosaccharides. *J. Cell Sci.* **109:**1617–1627

Rosales Fritz, V.M., Daniotti, J.L., Maccioni, H.J.F. 1997. Chinese hamster ovary cells lacking GM1 and GD1a synthesize gangliosides upon transfection with human GM2 synthase. *Biochim. Biophys. Acta Gene Struct. Expression* **1354:**153–158

Roseman, S. 1970. The synthesis of complex carbohydrates by multiglycosyltransferase systems and their potential function in intercellular adhesion. *Chem. Phys. Lipids* **48:**270–297

Roth, J., Taatjes, D.J., Weinstein, J., Paulson, J.C., Greenwell, P., Watkins, W.M. 1986. Differential subcompartmentation of terminal glycosylation in the Golgi apparatus of intestinal absorptive and goblet cells. *J. Biol. Chem.* **261:**14307–14312

Roth, J., Wang, Y., Eckhardt, A.E., Hill, R.L. 1994. Subcellular localization of the UDP-*N*-acetyl-D-galactosamine: Polypeptide *N*-acetylgalactosaminyltransferase-mediated O-glycosylation reaction in the submaxillary gland. *Proc. Natl. Acad. Sci. USA* **91:**8935–8939

Rottger, S., White, J., Wandall, H., Olivo, J.C., Stark, A., Bennett, E., Whitehouse, C., Berger, E., Clausen, H., Nilsson, T. 1998. Localization of three human polypeptide GalNAc-transferases in HeLa cells suggests initiation of O-linked glycosylation throughout the Golgi apparatus. *J. Cell Sci.* **111:**45–60

Sasai, K., Ikeda, Y., Tsuda, T., Ihara, H., Korekane, H., Shiota, K., Taniguchi, N. 2001. The critical role of the stem region as a functional domain responsible for the oligomerization and Golgi localization of N-acetylglucosaminyltransferase V - The involvement of a domain homophilic interaction. *J. Biol. Chem.* **276:**759–765

Schutze, M.-P., Peterson, P.A., Jackson, M.R. 1994. An N-terminal double-arginine motif maintains type II membrane proteins in the endoplasmic reticulum. *EMBO J.* **13:**1696–1705

Sekine, M., Hashimoto, Y., Suzuki, M., Inagaki, F., Takio, K., Suzuki, A. 1994. Purification and characterization of UDP-GlcNAc:IV3 beta Gal-Gb4Cer beta-1,6-GlcNAc transferase from mouse kidney. *J. Biol. Chem.* **269:**31143–31148

Simmons, A.D., Musy, M.M., Lopes, C.S., Hwang, L.Y., Yang, Y.P., Lovett, M. 1999. A direct interaction between EXT proteins and glycosyltransferases is defective in hereditary multiple exostoses. *Hum. Mol. Genet.* **8:**2155–2164

Sjoberg, E.R., Varki, A. 1993. Kinetic and spatial interrelationships between ganglioside glycosyltransferases and *O*-acetyltransferase(s) in human melanoma cells. *J. Biol. Chem.* **268:** 10185–10196

Sousa, V.L., Brito, C., Costa, T., Lanoix, J., Nilsson, T., Costa, J. 2003. Importance of Cys, Gln, and Tyr from the transmembrane domain of human alpha 3/4 fucosyltransferase III for its localization and sorting in the Golgi of baby hamster kidney cells. *J. Biol. Chem.* **278:**7624–7629

Stolz, J., Munro, S. 2002. The components of the *Saccharomyces cerevisiae* mannosyltransferase complex M-Pol I have distinct functions in mannan synthesis. *J. Biol. Chem.* **277:**44801–44808

Strous, G.J. 1986. Golgi and secreted galactosyltransferase. *CRC Crit. Rev. Biochem.* **21:**119–151

Ten Hagen, K.G., Fritz, T.A., Tabak, L.A. 2003. All in the family: the UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferases. *Glycobiology* **13:**1R–16R

van Vliet, C., Thomas, E.C., Merino-Trigo, A., Teasdale, R.D., Gleeson, P.A. 2003. Intracellular sorting and transport of proteins. *Prog. Biophys. Mol. Biol.* **83:**1–45

Varki, A. 1998. Factors controlling the glycosylation potential of the Golgi apparatus. *Trends Cell Biol.* **8:**34–40

Yamaguchi, N., Fukuda, M.N. 1995. Golgi retention mechanism of beta-1,4-galactosyltransferase. *J. Biol. Chem.* **270:**12170–12176

Yen, T.Y., Macher, B.A., Bryson, S., Chang, X., Tvaroska, I., Tse, R., Takeshita, S., Lew, A.M., Datti, A. 2003. Highly conserved cysteines of mouse core 2 beta 1,6 N-acetylglucosaminyltransferase I form a network of disulfide bonds and include a thiol that affects enzyme activity. *J. Biol. Chem.* **278:**45864–45881

Young, W.W. Jr., Lutz, M.S., Blackburn, W.A. 1992. Endogenous glycosphingolipids move to the cell surface at a rate consistent with bulk flow estimates. *J. Biol. Chem.* **267:**12011–12015

Yuan, Z., Teasdale, R.D. 2002. Prediction of Golgi Type II membrane proteins based on their transmembrane domains. *Bioinformatics.* **18:**1109–1115

Zhu, G., Allende, M.L., Jaskiewicz, E., Qian, R., Darling, D.S., Worth, C.A., Colley, K.J., Young, W.W. Jr. 1998. Two soluble glycosyltransferases glycosylate less efficiently in vivo than their membrane bound counterparts. *Glycobiology* **8:**831–840

Zhu, G., Jaskiewicz, E., Bassi, R., Darling, D.S., Young, W.W. Jr. 1997. beta1,4 N-acetylgalactosaminyltransferase (GM2/GD2/GA2 synthase) forms homodimers in the endoplasmic reticulum: a strategy to test for dimerization of Golgi membrane proteins. *Glycobiology* **7:**987–996