Glycobiology vol. 7 no. 1 pp. 1–13, 1997

MINI REVIEW

Golgi localization of glycosyltransferases: more questions than answers

Karen J.Colley

Department of Biochemistry, University of Illinois College of Medicine, 1819 West Polk Street M/C 536, Chicago, IL 60612, USA

The structures of cellular oligosaccharides are determined by a series of processing reactions catalyzed by Golgi glycosidases and glycosyltransferases. While there are subtle cell type differences in Golgi enzyme subcompartmentation, in general, glycosylation enzymes are localized within the Golgi cisternae in the same sequence in which they act to modify oligosaccharide substrates. The possibility that this enzyme subcompartmentation may control the types of oligosaccharides expressed by a cell has led to an interest in the signals and mechanisms directing enzyme localization in the Golgi cisternae. All glycosidases and glycosyltransferases characterized thus far have very little sequence homology that might suggest a common Golgi retention signal, but they do share a similar domain structure. They are all type II transmembrane proteins consisting of an amino terminal cytoplasmic tail, a signal anchor transmembrane domain, a stem region, and a large luminal catalytic domain. Their lack of sequence homology suggests that these proteins' Golgi retention signals are not linear amino acid sequences, but most likely involve general characteristics or conformations of larger protein domains. The peptide sequences required for Golgi retention of the N-acetylglucosaminyltransferase I (GlcNAcTI), β1,4-galactosyltransferase (GalT) and α2,6-sialyltransferase (ST) have been extensively studied. To do this, researchers created mutant and chimeric proteins, expressed these in tissue culture cells, and localized these proteins using immunofluorescence microscopy or immunoelectron microscopy. The cell surface expression of deletion mutants suggested that the deleted sequences were necessary for Golgi retention. Then, if these sequences were fused to a non-Golgi reporter protein and this chimeric or hybrid protein was retained in the Golgi, then these sequences were also sufficient for Golgi retention. Due to differences in reporter proteins used to construct these chimeric proteins, different cell types used for protein expression, different levels of protein expression, and different methods of cell surface protein detection, these experiments have led to somewhat confusing results. However, in general, it appears that the GalT relies primarily on its transmembrane domain for Golgi retention, while the GlcNAcTI and ST have requirements for their transmembrane regions, sequences flanking these regions, and luminal stem sequences. Based on these results, two potential Golgi retention mechanisms have been proposed and are now being tested. The observation that glycosyltransferase transmembrane domains are frequently sufficient for Golgi retention has led to the first of these models, the bilayer thickness model. This model proposes that the shorter transmembrane domains of Golgi proteins prevent them from entering cholesterol-rich transport vesicles destined for the plasma membrane, and that this leads to Golgi retention. The second of these models is supported by the role of multiple protein domains in the Golgi retention of some proteins. This model, the oligomerization/kin recognition model of Golgi retention, proposes that the formation of insoluble protein homo-oligomers or very large hetero-oligomers prevents protein movement into transport vesicles destined for later compartments. Initial work suggests that the bilayer thickness mechanism may play a role in the retention of some Golgi retained proteins; however, it is not the sole retention mechanism. Other evidence suggests that an oligomerization/kin recognition mechanism may be more common, but definitive proof for its general use in Golgi protein retention is lacking. More research is required to further elucidate the sequences and particularly the mechanisms of Golgi retention. In the future, we hope to be able to explain the cell type differences in glycosylation enzyme Golgi subcompartmentation, the different sequence requirements for the Golgi retention of the same enzyme in various cell types, and whether differences in glycosylation enzyme Golgi subcompartmentation change the types of oligosaccharides made by a cell.

Key words: glycosyltransferase/Golgi retention/oligomerization/lipid bilayer

Compartmentation of Golgi glycosylation enzymes

The specific Golgi compartmentation of the glycosidases and glycosyltransferases associated with protein and lipid glycosylation has functionally defined the different Golgi cisternae. Several groups have localized these enzymes and their products in the subcompartments of the Golgi using both subcellular fractionation of cellular membranes and immunoelectron microscopy (reviewed in Roth, 1987). Early work suggested an orderly compartmentation of glycosylation enzymes that mirrored the sequence of oligosaccharide chain modification (Kornfeld and Kornfeld, 1985; Roth, 1987). This early view suggested that the α-mannosidases I and II (MannI and MannII) were found in the cis and medial Golgi, the GlcNAcTI in the medial Golgi, the GalT in the trans Golgi, and the ST in the trans Golgi and trans Golgi network. This relatively strict compartmentalization of enzymes was thought to ensure the efficient biosynthesis of oligosaccharide structures by providing optimal contact between enzyme, glycoprotein substrate, and sugar nucleotide donor. In addition, this organization was presumed to segregate competing enzyme reactions and thus direct the structure of the oligosaccharides expressed by a cell.

With further investigation, it became apparent that many of these glycosylation enzymes overlap in localization and that

they demonstrate cell-type-specific Golgi subcompartmentation. The MannI and MannII enzymes, once believed to be localized within the cis and medial Golgi cisternae, respectively, have been found to overlap within the medial and trans Golgi of most cell types (Velasco *et al.*, 1993). In HeLa cells, the GlcNAcTI and MannII overlap in the medial and trans Golgi cisternae, while the GalT and ST overlap in the trans cisternae and trans Golgi network (Nilsson *et al.*, 1993a; Rabouille *et al.*, 1995). Most striking are the differences in glycosyltransferase localization in the absorptive columnar cells and adjacent goblet cells of the intestine. Roth and colleagues (Roth *et al.*, 1986; Taatjes *et al.*, 1988) demonstrated that the ST and the blood group A N-acetylgalactosaminyltransferase are restricted to the trans-most cisternae of the goblet cells. In contrast, these enzymes are found throughout the Golgi stack except for the first fenestrated cis Golgi cisternae in absorptive cells. In addition to Golgi staining, these enzymes were found in the mucin droplets of goblet cells and on the plasma membranes of both intestinal cell types. More recently, similar staining patterns for both the MannI and MannII enzymes in these intestinal cells have been documented (Velasco *et al.*, 1993). Cell surface staining has also been observed for the GalT in a variety of cell types (Pestalozzi *et al.*, 1982; Roth *et al.*, 1985a; Shaper *et al.*, 1985; Lopez *et al.*, 1991; Teasdale *et al.*, 1992; Evans *et al.*, 1993; Youakim *et al.*, 1994). This too appears to be cell type specific since recent studies by Taatjes *et al.* (1992) demonstrated that the GalT is restricted to the trans cisternae of the Golgi in bovine epithelial cells with no cell surface staining apparent except in a subpopulation of tracheal cells. How changes in glycosyltransferase compartmentation may affect the oligosaccharide structures synthesized within a cell is still uninvestigated and awaits further information on the intra-Golgi localization of specific sugar nucleotide donors and their translocators (Hirschberg, 1996) and an evaluation of whether an enzyme localized in a specific subcompartment is truly functional.

While current data demonstrates that the Golgi stack is the primary location of the terminal oligosaccharide processing enzymes in the majority of cell types, the presence of cell surface enzymes and soluble enzyme forms in body fluids raises questions concerning the role of enzymes in these locations (Roseman, 1970; Bartholomew *et al.*, 1973; Paulson *et al.*, 1977; Ratnam *et al.*, 1981; Strous, 1986). Generally, it is believed that the glycosyltransferases that reside outside the Golgi do not perform the transferase function due to a lack of sugar nucleotide donors. Consequently, their roles have to be limited to those that would involve their carbohydrate binding ability. Essentially, these enzymes would act as lectins. Soluble glycosyltransferases have long been thought to be either products of constitutive turn-over events or the acute phase response (Jamieson *et al.*, 1993). While they may act as circulating lectins, this possibility has gone virtually unstudied. In contrast, work by several groups has suggested that cell surface localized glycosyltransferases may be acting as lectin-like cell adhesion molecules (reviewed in Roseman, 1970; Strous, 1986; Shur, 1993). Shur and colleagues (Evans *et al.*, 1995) have shown that cell adhesion to and spreading on laminin substratum is dependent upon the expression of surface GalT in certain cells. Work performed by these investigators has also suggested that mouse sperm plasma membrane GalT is involved in sperm–egg interactions during fertilization and that it can participate in a signal transduction cascade that involves activation of a G protein complex (Shur, 1993; Gong *et al.*, 1995). The extent of GalT's participation in fertilization is unclear since other proteins, like sp56 (Cheng *et al.*, 1994) and a 95 kDa phosphotyrosine-containing protein (Leyton and Saling, 1989), have also been shown to bind to the ZP3 glycoprotein on the egg cell surface and these too appear to play significant roles in fertilization. To date, the roles of other cell surface glycosyltransferases have not been widely studied.

The demonstrated Golgi localization of the glycosyltransferases involved in the terminal processing of Asn-linked carbohydrate structures has made these enzymes attractive models for studying protein localization and subcompartmentation in this organelle. Three of these enzymes, the GlcNAcTI, GalT, and ST, have been used to extensively study the signals and mechanisms of Golgi retention. Researchers have found that while the transmembrane domains of these enzymes are clearly important for their retention, frequently their cytoplasmic and/or luminal sequences play accessory, or even independent roles, in the Golgi retention process. The nature of the glycosyltransferase sequences required for Golgi retention has led to two major hypotheses for the mechanism of this retention. The first hypothesis postulates that the length of the hydrophobic transmembrane domain is the driving force behind Golgi retention (bilayer thickness model, Bretscher and Munro, 1993; Masibay *et al.*, 1993), while the second hypothesis postulates that protein oligomerization leads to Golgi retention (oligomerization/kin recognition hypothesis (Machamer, 1991; Nilsson *et al.*, 1993b)). Below, I discuss what is known concerning the peptide signals required for Golgi retention of the GlcNAcTI, GalT, and ST, and how this information fits with these two models for Golgi retention. Several other reviews concerning the Golgi localization of proteins have recently been published (Shaper and Shaper, 1992; Machamer, 1993; Pelham and Munro, 1993; Gleeson *et al.*), and readers are encouraged to read these for comparable and contrasting views of this field.

Sequences required for the efficient Golgi retention of glycosyltransferases

Protein transport through the secretory pathway

Proteins that are localized within the secretory pathway, found at the cell surface, or secreted from the cell are synthesized with a cleavable signal peptide or an uncleavable signal anchor that directs the nascent protein cotranslationally to the cytoplasmic membrane of the ER (Walter and Johnson, 1994). As these nascent proteins are extruded through an aqueous channel into the lumen of the ER, they form disulfide bonds, are cotranslationally core-glycosylated on Asn residues, and fold and oligomerize with the help of chaperone proteins. Proteins destined for locations beyond the ER were originally believed to be transported in a "bulk flow" manner, only stopping at a particular subcellular location by virtue of a retention signal specific for that location (Pfeffer and Rothman, 1987). New data demonstrates that this initial hypothesis is only partially correct. First, reevaluation of the original data (Wieland *et al.*, 1987) suggests that ER to Golgi transport could be mediated by transport signals (Rothman and Wieland, 1996). Second, it is now clear that some concentration events occur as the proteins leave the ER (Balch *et al.*, 1994). Third, it has been demonstrated by the work of the Pelham and Peterson groups (reviewed in Rothman and Wieland, 1996) that both soluble and membrane associated ER proteins are retrieved from the intermediate compartment (salvage compartment, cis Golgi net-

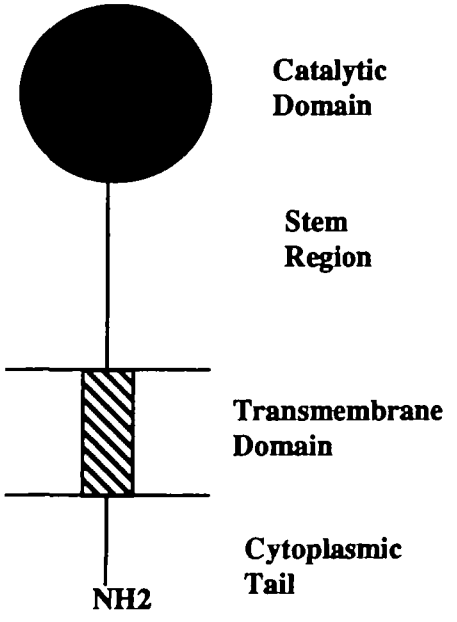
work) or later Golgi regions and brought back to the ER. Recycling of proteins is also apparent between the cell surface and the trans Golgi network (reviewed in Machamer, 1993; Gleeson *et al.*, 1994). Despite these many exceptions to the bulk flow hypothesis, movement through the Golgi appears to involve no concentration and can be essentially considered bulk flow (Orci *et al.*, 1986). The Golgi glycosyltransferases may be the only true examples of retained proteins since most studies have ruled out the recycling of these enzymes (Tang *et al.*, 1992; Wong *et al.*, 1992; Chapman and Munro, 1994; Teasdale *et al.*, 1994). However, one recent study by Hoe *et al.* (1995) does provide some evidence that GlcNAcTI may recycle between the cis and trans Golgi cisternae in the ldlD mutant CHO cell line.

Golgi localization of a coronavirus M glycoprotein

Early work by Machamer and Rose (1987) on the cis Golgi localization of a coronavirus M (E1) glycoprotein set the experimental tone for later work performed on glycosyltransferase Golgi localization. These researchers demonstrated that the first of the three transmembrane domains of the M glycoprotein is required for this protein's cis Golgi localization. Deletion of this region led to the bypass of the cis Golgi and this protein's cell surface expression. Later work by Machamer and colleagues (Swift and Machamer, 1991; Machamer *et al.*, 1993) showed that uncharged polar residues in the transmembrane domain of a M glycoprotein-VSV G chimera are important for Golgi retention, and suggested that their appropriate alignment on one face of the membrane spanning helix is crucial for Golgi retention. Later work by this laboratory showed that the formation of an insoluble oligomer correlated with the Golgi retention of this chimeric protein (Weisz *et al.*, 1993). Taken together, these results suggest that a transmembrane domain with appropriately aligned uncharged polar residues is able to mediate Golgi retention and that this retention involves the formation of insoluble homo-oligomers.

Golgi retention of glycosyltransferases: experimental variables

While it seemed likely that Golgi glycosyltransferases would also require sequences within their transmembrane domains for Golgi retention, their apparent lack of homology within these regions and throughout their entire sequences made it difficult to pinpoint any likely retention signals. The one common feature shared by all these enzymes is a type II membrane orientation. Each glycosyltransferase is comprised of an amino terminal cytoplasmic tail, a signal anchor domain that spans the membrane (referred to as the transmembrane domain throughout this article), followed by a luminal stem or stalk region, and a carboxy-terminal catalytic domain (Figure 1)(Paulson and Colley, 1989). To understand which glycosyltransferase sequences are necessary for Golgi retention, investigators first created glycosyltransferase mutants with deletions or alterations in these protein domains. Cell surface expression of these glycosyltransferase mutants indicated that the deleted or altered sequences were necessary for enzyme Golgi retention. These sequences were next fused to reporter proteins to determine whether they were also sufficient for Golgi retention of non-Golgi proteins. Surprisingly, those sequences found to be necessary for complete Golgi retention were not always sufficient for the retention of reporter proteins and visa versa. This kind of situation was observed in cases where there is more than one independent Golgi retention region in an enzyme and/or where several regions of a protein are required for com-



GlcNAcTI	GalT	ST
Not analyzed.	Not analyzed.	Not required.
Retention region. Required for interaction with MannII	Possible accessory role in retention?	Probable independent retention region.
Retention region.	Primary retention region. Length or characteristics important?	Retention region with flanking sequences, length not sequence important.
Retention region.	Possible accessory role in retention?	Not required.

Fig. 1. Glycosyltransferase domain structure and general Golgi retention requirements. Glycosyltransferases are type II transmembrane proteins that consist of an amino-terminal cytoplasmic tail, a signal anchor transmembrane domain followed by a stem or stalk region, and a large carboxy-terminal catalytic domain. The Golgi retention requirements for the enzymes listed in the table are purely general, and the reader should be aware that these may vary in different cell types.

plete Golgi retention. In addition, several variables in experimental design have complicated the interpretation of data and led to different conclusions concerning the importance of glycosyltransferase sequences in Golgi retention. These experimental variables include (1) the use of chimeric proteins constructed from a wide variety of reporter proteins, (2) differences in localization of proteins expressed in high level transient and low level stable expression systems (Teasdale *et al.*, 1994; Tang *et al.*, 1995), (3) differences in localization of proteins in different cell types (Teasdale *et al.*, 1994; Tang *et al.*, 1995), and (4) the use of biochemical and microscopic detection methods with a wide range of sensitivity.

Possibly the greatest conceptual problem encountered in all these studies is the initial assumption that deletions or alterations within a protein sequence only locally influence the protein structure at that site. It is more likely that many of the changes made within these proteins more globally alter the conformation of the protein and thus its localization. This could be particularly important in light of a recent possibility suggested by the work of Low and colleagues (Low *et al.*, 1994, 1995). These researchers and others (Low *et al.*, 1994, 1995; Wahlberg *et al.*, 1995 and references therein) have demonstrated that slightly altered plasma membrane proteins, or chimeric proteins made exclusively from plasma membrane and secretory proteins, are retained or retarded in the Golgi. They suggest that movement within the Golgi and from the trans Golgi network to the cell surface may be "gated" or signal dependent and that these Golgi retained chimeras or mutant cell surface proteins lack signals or conformations permissive for forward movement. In interpreting the experiments discussed below, we therefore must ask whether Golgi retention has been conferred to reporter proteins by adding sequences responsible for that retention, or whether the investigator has inadvertently created a protein that does not move beyond the Golgi due to a loss of transport competence, and whether these two situations are really different.

Galactosyltransferase: does the transmembrane domain play the major role in Golgi retention?

The GalT is predominantly localized in the trans cisternae of the Golgi (Berger *et al.*, 1981; Roth and Berger, 1982; Berger and Hesford, 1985; Bergeron *et al.*, 1985; Taatjes *et al.*, 1987, 1992; Russo *et al.*, 1992; Rabouille *et al.*, 1995), and also has been found in the trans Golgi network of some cells (Geuze *et al.*, 1985; Rabouille *et al.*, 1995). Work from several groups has demonstrated that its transmembrane domain is primarily responsible for its Golgi retention (Nilsson *et al.*, 1991; Aoki *et al.*, 1992; Teasdale *et al.*, 1992, 1994; Masibay *et al.*, 1993; Yamaguchi and Fukuda, 1995); however, some data suggests that other regions, including its cytoplasmic tail, may be involved in the retention process (Nilsson *et al.*, 1991; Evans *et al.*, 1993). Initial experiments demonstrated that the GalT cytoplasmic tail and transmembrane domain are able to retain reporter proteins such as pyruvate kinase and the lip31 invariant chain in the Golgi (Nilsson *et al.*, 1991; Russo *et al.*, 1992). Teasdale *et al.* (1992, 1994) provided evidence that the GalT transmembrane domain is necessary and sufficient for Golgi retention. They found that the GalT transmembrane domain alone was sufficient for retention of ovalbumin in the Golgi of murine L cells and that the replacement of this transmembrane domain with that of the transferrin receptor (TfR) resulted in predominant cell surface expression of the hybrid protein in Cos cells. Further analysis of GalT Golgi retention by Masibay *et al.* (1993) supported the idea that the GalT transmembrane domain is required for Golgi retention and demonstrated that different glycosyltransferases have different requirements for the Golgi retention. They showed that the tail and transmembrane domain of GalT could be replaced with the tail and transmembrane domain of the α1, 3GalT, but not with the tail and transmembrane domain of the ST. Only after the ST tail, transmembrane domain plus 18 amino acids of the stem region were added, was a protein synthesized, imported into the ER, and ultimately Golgi localized. These results emphasize the potential differences between the sequence requirements for GalT and ST Golgi retention and suggested that, while the transmembrane domain plays a primary role in GalT Golgi retention, the ST has more extensive sequence requirements for this process (see below).

To determine whether specific GalT transmembrane sequences were required for Golgi retention, Aoki *et al.* (1992) made a series of transmembrane domain replacements and showed that the replacement of Cys-Ala-Leu-His-Leu in the amino-terminal portion of the GalT transmembrane domain, or mutations of Cys_{29} and His_{32} in this region, led to an increase in Cos cell surface expression of a GalT-αhCG chimera. However, including these amino acids in the transmembrane domain of the TfR was not sufficient to confer Golgi retention to the hybrid protein. Similarly, Nilsson *et al.* (1991) showed that replacement of the amino terminal 10 amino acids of GalT transmembrane domain (including Cys_{29} and His_{32}) with transmembrane sequences of the Iip31 invariant chain still allowed predominant Golgi retention of the GalT-Iip31 chimera in HeLa cells, although a small amount of surface expression was observed. Taken together these observations again suggest that Golgi retention requirements differ in different cell types and also highlight the possibility that small changes or larger replacements can influence the folding of a region and its function in retention. Work by Masibay *et al.* (1993) also suggested the latter possibility. These researchers found that insertion of sequences and/or alteration of uncharged polar residues in the GalT transmembrane domain increased cell surface expression of mutant GalT proteins in Cos cells. From the data described above, it seems questionable that specific sequences within the transmembrane domain are required for GalT Golgi retention. More likely, the general characteristics of the transmembrane domain, such as the hydrophobic length of this region or the presentation of uncharged polar amino acids, are responsible for Golgi retention.

While most experiments have demonstrated a primary requirement for the GalT transmembrane domain in Golgi retention, some data suggest that other sequences may be important in the retention process depending upon the cell type in which the protein is expressed. Teasdale *et al.* (1994) demonstrated that the replacement of the transmembrane domain of the GalT with that of the TfR leads to predominant cell surface localization in transiently expressing Cos cells. However, stably expressing murine L cells retain a significant amount of this hybrid protein in the Golgi. These researchers concluded that while the transmembrane domain of the GalT was clearly important for retention, other sequences must play a role in the retention process. Nilsson *et al.* (1991) showed that deleting most of the GalT cytoplasmic tail sequences led to more cell surface expression of a GalT-dipeptidyl peptidase IV (D4) chimera in HeLa cells. Interestingly, this requirement for cytoplasmic sequences can be fulfilled by those of a plasma membrane protein. In the intact GalT protein, however, deletions of

19 amino acids (Aoki *et al.*, 1992) or of the first 13 amino acids of the cytoplasmic tail (Russo *et al.*, 1992; Teasdale *et al.*, 1992; Masibay *et al.*, 1993) had no effect on Golgi retention in most other cell types. The differences in cells used for expression and/or the absence of the GalT luminal sequences in the chimeric proteins of Nilsson *et al.* (1991) could explain the differences in results observed by these researchers and others (Aoki *et al.*, 1992; Russo *et al.*, 1992; Teasdale *et al.*, 1992; Masibay *et al.*, 1993). Although inconclusive, these results may suggest a contributing role for both cytoplasmic and luminal sequences in efficient GalT Golgi retention in some cell types.

Paradoxically, some evidence suggests that the naturally occurring, longer GalT cytoplasmic tail plays the major role in the cell surface expression of this enzyme in certain cell types. Two forms of the GalT are encoded by the bovine and murine GalT genes (Russo *et al.*, 1990). The short form of the GalT possesses an 11 amino acid cytoplasmic tail, while the long form has an additional 13 amino acid extension of this region. The dual Golgi/plasma membrane localization of the GalT in some cells raised the possibility that the cytoplasmic tails of the two forms specify their Golgi and cell surface locations. Russo *et al.* (1992) showed that the long and short forms of the GalT were both retained in the Golgi of stably expressing CHO cells. This result was also confirmed by Masibay *et al.* (1993) using a transient high level expression system in Cos-7 cells. However, work by Shur and colleagues (Lopez *et al.*, 1991; Youakim *et al.*, 1994; Evans *et al.*, 1995) demonstrated that the form of the enzyme with the longer tail is found both in the Golgi and at the cell surface of stably expressing F9 embryonal carcinoma cells and Swiss 3T3 fibroblasts, while the form with the shorter tail is retained in the Golgi of these cells. It is likely that the differences in localization of the two GalT forms are related to differences in protein trafficking and retention processes in the different cell types analyzed. This observation makes it even more important to understand the differences in cisternal environments in different cell types and how this effects the Golgi retention mechanisms used by these cells.

In summary, the Golgi retention of the GalT primarily depends on its transmembrane domain. Current evidence suggests that the hydrophobic length and/or the presentation of uncharged polar amino acids in this region may be more important than the presence of specific sequences in conferring Golgi retention. Some data suggests that other regions may also be required for efficient retention. The role of the GalT cytoplasmic tail sequences is complicated. In the presence of the GalT transmembrane domain but without a significant cytoplasmic tail, chimeric proteins exhibit some cell surface expression; however, addition of cytoplasmic sequences from a plasma membrane protein will allow complete Golgi retention. These results suggest that the presence of cytoplasmic tail sequences of sufficient length may play an accessory role in the retention process. In contrast, the naturally occurring 24 amino acid cytoplasmic tail has been associated with cell surface expression of the Galt in a few cell types, while the shorter 11 amino acid tail has been associated with Golgi retention of the enzyme in these cells. In CHO and Cos cells, however, GalT proteins possessing the longer tail show no increase in cell surface expression. Finally, while the stem region is not required for retention of chimeric proteins or the GalT catalytic domain, its presence appears to override the absence of the cytoplasmic tail sequences, suggesting that it too may play an accessory role in Golgi retention.

N-Acetylglucosaminyltransferase I: are all regions of the enzyme required for efficient retention?

The GlcNAcTI has been localized within the medial or medial and trans Golgi cisternae of different cell types (Dunphy *et al.*, 1985; Burke *et al.*, 1992; Rabouille *et al.*, 1995). After initial experiments showed that the GlcNAcTI transmembrane region played a primary role in Golgi retention, it became clear that the stem region was equally important for full Golgi retention and may act as an independent retention region. Initially, Burke *et al.* (1992) demonstrated that the GlcNAcTI transmembrane domain and flanking sequences were sufficient to retain ovalbumin in the Golgi of transiently expressing Cos-1 cells or stably expressing murine L cells. The work of Tang *et al.* (1992) supported a major role for the GlcNAcTI transmembrane domain in Golgi retention in stably expressing MDCK cells, but also suggested that the enzyme's stem region contributes to the retention process. These workers showed that the tail and transmembrane domain of the GlcNAcTI, or the transmembrane domain alone, allowed Golgi retention of D4 in the Golgi; however, a significant proportion of both chimeric proteins were still found at plasma membrane. Including increasing lengths of the GlcNAcTI stem region in the hybrid protein resulted in decreased cell surface staining. These results suggested that the GlcNAcTI transmembrane domain plays a predominant role in Golgi retention and that the luminal stem sequences increase the efficiency of this retention. Definitive work by Burke *et al.* (1994) elegantly demonstrated that all three domains of the GlcNAcTI are important for Golgi retention in stably transfected murine L cells. Using immunofluorescence microscopy and FACS analysis of chimeras containing domains from GlcNAcTI, ovalbumin and the TfR, they found that replacing the GlcNAcTI luminal region, transmembrane domain, or tail resulted in a 1.4- to 2-fold increase in cell surface staining, while replacing the tail plus transmembrane domain or the tail plus luminal region resulted in a 5.4- to 6.0-fold increase in cell surface staining. These results suggested a role for all three GlcNAcTI regions in Golgi retention and support an oligomerization-based retention mechanism that could involve all GlcNAcTI domains.

Recent results from Warren and colleagues (Nilsson *et al.*, 1996) call into question a primary role for the GlcNAcTI transmembrane domain in Golgi retention. Work by these researchers suggests that the stem region of the GlcNAcTI is necessary and sufficient for Golgi retention. They demonstrate that charged amino acids in the GlcNAcTI stem region are required for the interaction of GlcNAcTI with MannII and its resulting Golgi retention in HeLa cells. They also suggest that the GlcNAcTI transmembrane domain is not necessary for Golgi retention because replacement of this region with 19, 23, or 27 leucine residues does not alter Golgi retention. However, these transmembrane domain changes were made in the presence of the GlcNAcTI stem region and catalytic domain. It is probable that the GlcNAcTI stem region serves as an independent retention signal in HeLa cells and is sufficient for Golgi retention in the absence of the transmembrane sequences. This then could explain why changes in the transmembrane domain have no major effects on Golgi retention when these stem sequences are present. Interestingly, the stacked structure of the Golgi apparatus was disrupted in cells expressing GlcNAcTI proteins containing polyleucine transmembrane domains, suggesting that the GlcNAcTI transmembrane domain may also play an important role in Golgi structural integrity.

Again, somewhat conflicting results from different laboratories using different cell expression systems make it difficult to conclusively state which sequences are required for GlcNAcTI Golgi retention. Work by Gleeson and colleagues (Burke *et al.*, 1994) clearly demonstrates a role for all GlcNAcTI domains in its Golgi retention in murine L cells. However, Warren and colleagues (Nilsson *et al.*, 1996) suggest that the GlcNAcTI stem region alone is sufficient for its kin recognition and Golgi retention in HeLa cells, while the enzyme's transmembrane domain solely influences stability of the Golgi stacks in these cells. As mentioned above, the results of Nilsson *et al.* (1996) do not necessarily negate the contributions of the GlcNAcTI transmembrane domain to Golgi retention, especially if one considers that the analysis of the transmembrane domain was done in the presence of the GlcNAcTI stem region, a region that seems to act as an independent retention signal in HeLa cells. These data are clearly examples of how cell type differences in retention requirements, and redundant Golgi retention signals in the same enzyme, can lead to different conclusions concerning the role of distinct protein domains in Golgi retention.

Sialyltransferase: independent Golgi retention signals in the transmembrane and stem regions?

The ST has been localized in the trans cisternae of the Golgi and trans Golgi network of hepatocytes and intestinal goblet cells, while it is spread throughout the Golgi stack in intestinal absorptive cells (Berger and Hesford, 1985; Roth *et al.*, 1985b, 1986; Roth, 1987; Taatjes *et al.*, 1987, 1988; Berger *et al.*, 1995; Rabouille *et al.*, 1995). The ST appears to have the most unusual set of overlapping requirements for Golgi retention since several different regions and combinations of sequences seem to be sufficient for this process. Using different experimental systems, researchers have shown that the ST transmembrane domain plus flanking sequences, the flanking sequences appropriately spaced by a transmembrane region of specific length, and the ST stem region alone, all appear to constitute independent Golgi retention signals. Initial work demonstrated that the soluble catalytic domain of the ST is rapidly secreted from cells and implied that sequences required for Golgi retention would be found in the cytoplasmic tail, transmembrane domain and/or stem region (Colley *et al.*, 1989). Later, several studies suggested that the transmembrane domain of the ST, and sequences flanking this region, are sufficient for Golgi retention (Munro, 1991; Dahdal and Colley, 1993; Tang *et al.*, 1995). Using ST-D4 chimeras, Wong *et al.* (1992) initially concluded that the ST transmembrane domain alone was sufficient for Golgi retention of D4 reporter sequences when stably expressed in MDCK cells. Later this group found that the same chimeric protein expressed in CHO cells was predominantly targeted to lysosomes and Golgi retention was only achieved when ST stem sequences were present (Tang *et al.*, 1995). Dahdal and Colley (1993) showed that the ST transmembrane domain and flanking sequences were sufficient for retention of the ST catalytic domain in a Cos cell transient overexpression system. Munro (1991) demonstrated that D4-ST-lysozyme chimeras containing the ST transmembrane domain alone exhibited increased Golgi retention in Cos cells; however, full Golgi retention was only observed when the ST transmembrane domain plus flanking cytoplasmic and stem sequences were present. So while the ST transmembrane domain alone may be sufficient for Golgi retention in MDCK cells, the ST transmembrane region and flanking sequences are clearly required for Golgi retention in Cos and CHO cells.

The ability of the ST transmembrane region and flanking sequences to allow efficient Golgi retention also appears to be dependent on the nature of the luminal reporter sequences. While the ST transmembrane domain and flanking sequences are sufficient for retention of the ST catalytic domain in the Golgi, chimeric proteins consisting of the ST cytoplasmic tail and transmembrane domain fused to the stalk and head regions of the influenza neuraminidase or the ectodomain of TfR were found at the cell surface when expressed in CHO cells or Cos-1 cells (Colley *et al.*, 1992; Dahdal and Colley, 1993). When two luminal lysine residues were added to the ST-neuraminidase chimera, Golgi retention of this chimera increased, but was not complete (Dahdal and Colley, 1993). In addition, the ST-TfR chimera contains a flanking luminal lysine residue contributed by the TfR sequences and still did not exhibit significant Golgi retention. These results and those above suggest that the transmembrane domain of the ST plus flanking sequences are sufficient for Golgi retention; however, the efficiency of this retention may depend on both luminal sequences of the protein and the cells in which these proteins are expressed.

Work done by Munro (1991) and Colley and colleagues (Colley *et al.*, 1992; Dahdal and Colley, 1993) demonstrated that no specific sequences are required in the ST transmembrane domain for efficient retention, especially in the presence of appropriately spaced cytoplasmic and luminal flanking sequences and/or the ST stem region. Sequentially replacing 4–5 amino acids along the entire length of the ST transmembrane domain with sequences from the influenza neuraminidase transmembrane domain did not alter the Golgi retention of an intact ST protein or a ST protein lacking all but the first five amino acids of its stem region (Colley *et al.*, 1992; Dahdal and Colley, 1993). Likewise, 17 leucine residues could replace the 17 amino acid ST transmembrane domain in a lysozyme chimera containing the ST tail and the first 18 amino acids of the ST stem sequence (Munro, 1991). However, the same construct with a 17 leucine transmembrane domain plus luminal sequences derived from D4, was found at the cell surface. These results demonstrate that appropriately spaced ST cytoplasmic (LysLysLys) and stem sequences (LysLysGlySerAsp . . .) are sufficient for Golgi retention.

What really determines the correct spacing of the ST sequences flanking the membrane is the actual length of the hydrophobic transmembrane region. Results from Munro's laboratory show that the length of the transmembrane domain is crucial for Golgi retention of ST hybrid proteins (Munro, 1991, 1995b). While a ST-lysozyme chimera with a 17 leucine transmembrane domain and ST flanking sequences was retained in the Golgi, a similar chimeric protein with a 23 leucine transmembrane domain was found in increased amounts at the cell surface, suggesting that the length of the ST transmembrane domain (and thus the spacing of cytoplasmic and stem sequences) is crucial for Golgi retention. In addition, incremental increases in the length of the ST transmembrane domain by insertion of 1–9 hydrophobic amino acids also resulted in increased cell surface expression of similar ST-lysozyme chimeras, while the decrease in the length of a plasma membrane protein's transmembrane domain led to its increased retention in the Golgi (Munro, 1995b). Interestingly, Dahdal and Colley (1993) found that replacement of the 17 amino acid transmembrane domain of the ST with either 29 or 23 amino acids of the neuraminidase transmembrane domain still al-

lowed efficient Golgi retention. Comparison of these constructs and their localization suggests that the presence of the entire ST stem and catalytic domain is able to overcome any increase in the length of the ST transmembrane domain and allow retention in the Golgi. These results imply that the ST stem or catalytic domain, or both of these regions, may act as independent Golgi retention signals.

The ST stem region as an independent Golgi retention signal was first suggested by Colley *et al.* (1992), who found that a mutant protein consisting of only the ST stem and catalytic domain (signal cleavage-ST) was transiently retained in the Golgi, while the soluble ST catalytic domain was rapidly secreted. Interestingly, this behavior was markedly different from that of a similar GalT protein constructed by Teasdale *et al.* (1992), in which the tail and transmembrane domain of the GalT were replaced with the cleavable signal peptide of influenza hemagglutinin. This mutant GalT protein was rapidly secreted from cells. It was possible that the signal cleavage-ST protein was "docking," through interactions in its luminal domain, to preexisting retained molecules in the Golgi, thus leading to its retention (Gleeson *et al.*, 1994). In contrast, the analogous GalT protein could not make similar contacts because its transmembrane domain was missing, and therefore was secreted rapidly. These differing results again suggest that these two Golgi enzymes may have different requirements and mechanisms of retention (see also Masibay *et al.*, 1993). While Munro (1991) found that the first 18 amino acids of the ST stem were not sufficient by themselves to retain a D4-lysozyme chimera in the Golgi, recent results from our laboratory confirm our initial belief that the entire stem region is an independent signal for Golgi retention. In an $NA_{T+SA}ST_{ST+CAT}$ chimera, the tail and 23 amino acids of the NA transmembrane domain were fused to the ST stem and catalytic domain. This protein was efficiently retained in the Golgi when expressed at high levels in Cos-1 cells (M. Tigue, J. Ma, and K. Colley, unpublished results). In contrast, a second chimera, $NA_{T+SA+ST}ST_{CAT}$, consisting of the NA tail, transmembrane domain and stalk region fused to the ST catalytic domain, was efficiently transported to the cell surface of these cells (J. Ma and K. Colley, unpublished results). These results suggest strongly that the ST stem region is indeed an independent Golgi retention region while the ST catalytic domain alone does not possess retention capability.

Together these data show that the ST has a number of regions that can independently allow Golgi retention. First, the ST transmembrane domain and flanking sequences are sufficient for retention. Second, appropriately spaced membrane flanking sequences themselves are also sufficient for retention, this being a function of the length of the transmembrane domain. And third, ST stem sequences alone also appear to constitute another type of Golgi retention signal. The variety of sequences that are sufficient for Golgi retention also suggest that more than one retention mechanism may be at work in the Golgi localization of this enzyme. This possibility may be able to explain its dual localization in the trans and trans Golgi network in many cell types and its differential localization in absorptive and goblet cells of the intestine.

Mechanisms of retention

Recently, two predominant hypotheses concerning the mechanism of Golgi protein retention have dominated the literature. First is the oligomerization/kin-recognition hypothesis, which suggests oligomerization of Golgi proteins leads to their inability to enter transport vesicles destined for the next secretory pathway compartment and thus causes their retention in specific cisternae (Machamer, 1991; Nilsson *et al.*, 1993b). Second is the bilayer thickness model, which states that the relatively short transmembrane domains of Golgi proteins do not allow them to enter into cholesterol-rich transport vesicles destined for later compartments and the plasma membrane (Bretscher and Munro, 1993; Masibay *et al.*, 1993). In both models, retention would depend on the specific microenvironment of one or more Golgi cisternae. Below, I summarize the evidence for each of these mechanisms and speculate as to the extent of their contributions to the Golgi retention process.

Oligomerization/kin recognition model of Golgi retention

Early on, Machamer (1991) suggested that proteins may form oligomers in response to the different microenvironments found in the Golgi cisternae. She initially suggested that the composition of the lipid bilayer may be the major driving force for this oligomerization, because at that time, the transmembrane domains of the Golgi proteins investigated seemed to constitute their sole retention signals. This was strengthened by the differences in lipid composition and cholesterol concentration found in each organelle (Zambrano *et al.*, 1975; Orci *et al.*, 1981). In addition, a mechanism such as this could explain a gradient of glycosylation enzymes throughout the Golgi complex and the differential localization of specific glycosyltransferases in different cell types. Further research demonstrated that other domains of glycosyltransferases do participate in their Golgi retention and suggested even more strongly that multiple interactions through several protein domains could contribute to this type of oligomerization-based retention process. The kin recognition hypothesis of Warren and colleagues (Nilsson *et al.*, 1993b) extended this retention via oligomerization idea to suggest that enzymes residing in the same Golgi cisternae could form hetero-oligomers and this would prevent their exit from a particular cisternae because of an inability of the very large oligomer to enter into transport vesicles. These researchers (Nilsson *et al.*, 1994) believe that hetero-oligomerization is a requirement for an oligomerization-based retention mechanism due to (1) the low abundance of the individual glycosylation enzymes, (2) the even distribution of these enzymes throughout a cisternae, and (3) the large size of oligomer required to prevent entry into transport vesicles. Now with the realization that several enzymes overlap in localization throughout the Golgi cisternae, the formation of hetero-oligomers has become more feasible, and if it occurs, could provide some functional advantage to the glycosylation system of a cell by increasing the efficiency of sequential enzyme reactions and segregating competing reactions.

With this hypothesis in mind, Nilsson *et al.* (1994) have provided evidence for complex formation between two medial Golgi enzymes, the GlcNAcTI and the MannII. They demonstrated that coexpression of the wild type GlcNAcTI with an ER-retained form of MannII leads to significant ER retention of the GlcNAcTI, implying stable complex formation between the two enzymes. The reverse experiment led to the same results. In contrast, GalT, a predominantly trans Golgi protein, was not significantly retained by either ER-retained medial Golgi enzyme. Hetero-oligomerization of the GlcNAcTI and GlcNAcTII has also been suggested by the co-immunoprecipitation of experiments of Gleeson and colleagues (P.A. Gleeson, personal communication). These results suggested

that two medial Golgi enzymes could form kin complexes and that this may be the mechanism of their Golgi retention. Later work by Munro (1995b) showed that the luminal sequences of GlcNAcTI were required for its interaction with MannII and that a similar ER interaction between two later Golgi enzymes, the GalT and ST, was not detectable. Warren and coworkers (Nilsson *et al.*, 1996) recently suggested that charged residues in the stem region of the GlcNAcTI are required for the interaction with MannII and that this allows the retention of GlcNAcTI in the Golgi. Unfortunately, the regions of MannII that are required for its Golgi retention have not been studied. One puzzling aspect of this co-retention assay is the ability of these Golgi enzymes to form complexes in the ER environment. If the specific microenvironment of a Golgi cisterna leads to hetero-oligomerization and retention of resident enzymes, then one would not expect oligomerization in the ER. Consequently, the lack of GalT-ST complex formation in the ER could be explained by the inappropriate environment found in this compartment. However, another possibility that would explain MannII-GlcNAcTI complex formation in the ER, is that a certain concentration of an enzyme or enzymes must be achieved before oligomerization, and thus retention, can occur. If this is the case, then this ER co-retention experiment may be fulfilling this first essential step in the oligomerization process.

While hetero-oligomerization of Golgi glycosylation enzymes is certainly attractive from both a Golgi retention standpoint and a functional standpoint, homo-oligomerization of enzymes could also lead to retention. It is possible that the formation of homo-oligomers would change the characteristics of the enzyme sufficiently as to lead to an interaction with the lipid bilayer or preexisting luminal protein structures that would not occur with enzyme monomers. This interaction would in turn lead to retention. Work by Machamer and colleagues (Weisz *et al.*, 1993) has suggested that the formation of insoluble homo-oligomers of a coronavirus M glycoprotein-VSV G protein chimera correlate with its retention in the cis Golgi. Similarly, Schweizer *et al.* (1994) found that the formation of insoluble homo-oligomers involving interactions of all three protein domains of the p63 protein led to this protein's retention in the ER-Golgi intermediate compartment.

Have homo-oligomers been found for Golgi glycosylation enzymes? Crosslinking experiments performed by Teasdale *et al.* (1994) suggest the presence of GalT homo-oligomers, while the work of Yamaguchi and Fukuda (1995) suggests that homo-dimers and possibly homo-oligomers of a GalT chimera correlate with its Golgi retention. Ma and Colley (1996) have recently found that one-third of the ST in liver Golgi membranes is a disulfide-bonded dimer. This form of the enzyme is preferentially pelleted when Golgi membranes are solubilized in nonionic detergent at low pH (pH 6.4), suggesting that it may exist in an insoluble complex in the lower pH environment of the late Golgi (Ma and Colley, unpublished results). Little to no ST monomer form is found in this low pH, high speed pellet suggesting that dimerization actually may alter the characteristics of the ST. *In vivo,* the ST may be in fact a combination of noncovalently and convalently associated dimers as suggested by our work and the radiation target inactivation studies of Fleischer *et al.* (1993). Upon solubilization of membranes with nonionic detergents, the noncovalently associated dimers may fall apart and more easily dissociate from putative oligomers, while the disulfide-bonded dimers remain intact and more tightly associated. It is therefore possible that, *in vivo,* ST dimers of any sort would possess characteristics that favor insoluble oligomer formation.

Another corollary to Warren and colleagues' kin recognition hypothesis is that cytoplasmic anchoring of hetero-oligomers will enhance retention by ensuring that the complexes do not move into transport vesicles (Nilsson *et al.*, 1993b). Cluett and Brown (1992) provided evidence for a cytoskeletal network that stabilized the stacking of Golgi cisternae by biochemically and microscopically demonstrating the existence of proteinaceous crosslinks between adjacent Golgi cisternae. Additionally, early work on the solubilization of the MannII enzyme suggested that it may be associated with a cytoplasmic matrix (Tulsiani *et al.*, 1977). Recently, the cytoplasmic matrix associated with the medial Golgi enzymes, MannII and GlcNAcTI, has been isolated by Slusarewicz *et al.* (1994). Extensive evidence for cytoplasmic matrices associated with cis and trans/trans Golgi network glycosylation enzymes has not been found. However, the data of Weisz *et al.* (1993) suggest that the cytoplasmic tail of the cis Golgi-retained coronavirus M glycoprotein-VSV G chimera may interact with an actin-associated cytoskeleton matrix, while Yamaguchi and Fukuda (1995) have co-immunoprecipitated α- and β-tubulins with a Golgi retained GalT-TfR chimera suggesting that interactions with cytoskeletal proteins may play a role in this chimera's Golgi retention.

Bilayer thickness model of Golgi retention

The bilayer thickness model of Golgi protein retention was first suggested by both Bretscher and Munro (1993) and Masibay *et al.* (1993) in response to the finding that increasing the length of the transmembrane domain of Golgi retained proteins led to an increase in their cell surface expression. Comparisons revealed that the length of Golgi proteins' transmembrane domains were on average 5 amino acids shorter than those of plasma membrane proteins (Masibay *et al.*, 1993; Munro, 1995a). It is also known that the concentration of cholesterol increases from the ER, through the Golgi and to the plasma membrane with the highest levels found at the plasma membrane (Orci *et al.*, 1981). In addition, other researchers (Levine and Wilkins, 1971; Nezil and Bloom, 1992) demonstrated that increasing the amount of cholesterol in an egg phosphatidylcholine membrane increased the width of the membrane and suggested that the width of organellar membranes might increase with increasing cholesterol concentrations. Based on this *in vitro* data, the plasma membrane would be expected to be the thickest membrane along the secretory pathway. Short transmembrane domains containing a greater number of amino acids with large extended side chains (like Phe), as found in many Golgi proteins, would tend to exclude these proteins from membrane domains rich in cholesterol, such as the plasma membrane and transport vesicles moving to this region (Bretscher and Munro, 1993). This model could also account for the formation of a gradient of Golgi enzymes with progressively longer transmembrane domains and for differential localization of Golgi enzymes in different cell types depending on differences in the steepness of the cholesterol gradient within the cisternal membranes.

Lengthening of transmembrane domains of wild type and chimeric glycosyltransferases has led to increased cell surface expression of these proteins, suggesting that the bilayer thickness mechanism may contribute to the Golgi retention of some of these proteins. Munro (1991) showed that replacing the transmembrane domain of the ST with the larger transmem-

brane domain of D4 increased the cell surface expression of this protein. He also demonstrated that insertion of hydrophobic amino acids into the transmembrane domain of a ST-lysozyme chimera led to increasing amounts of this protein expressed at the cell surface and that shortening the length of the transmembrane domain of D4, a plasma membrane protein, increased its Golgi retention (Munro, 1995b). Masibay *et al.* (1993) made a series of Ile insertions in the transmembrane domain of the wild type GalT protein and found that these increased cell surface expression of this enzyme. These researchers concluded that the increased length of the GalT transmembrane domain disrupted its Golgi retention; however, they acknowledged that these insertions could have also altered the folding of the transmembrane domain and the presentation of amino acids required for retention. Earlier work by Swift and Machamer (1991) also demonstrated that insertion of two Ile residues into the 22 amino acid transmembrane domain of the coronavirus M glycoprotein-VSV G chimera led to increased cell surface expression. However, they did not view this as an increase in transmembrane domain length, but instead saw these insertions as altering the correct presentation of uncharged polar residues required for oligomerization and thus Golgi retention.

It is unclear whether insertion of hydrophobic sequences into a transmembrane domain lengthens the hydrophobic region or alters of the folding of the transmembrane domain, and thus the presentation of specific amino acids, or both. For the ST, where it has been demonstrated that the sequences of the transmembrane domain are not crucial for retention if the flanking sequences and/or stem region is present (Munro, 1991; Dahdal and Colley, 1993), one would expect that insertion of amino acids in the ST transmembrane domain will not change ST Golgi retention by altering the presentation of a specific series of transmembrane amino acids (Munro, 1995b). However, these insertions could have altered the folding or presentation of membrane flanking regions that seem to be important for Golgi retention (Munro, 1991; Dahdal and Colley, 1993). This may be particularly important if the lysines flanking the ST transmembrane domain are required to interact with phospholipid head groups for efficient retention to occur. In the case of the GalT, where either the length of the transmembrane domain or aligned uncharged polar residues may be important for Golgi retention, it is impossible to determine exactly which characteristics of the region have been altered leading to loss of retention. Consequently, it is difficult to assess how lengthening the transmembrane region of a protein disrupts its Golgi retention when it is unclear what characteristics of this region are important for retention.

Does the length of the transmembrane domain and retention via the bilayer thickness mechanism play a major role in the Golgi retention of the wild type glycosyltransferases? Results of Masibay *et al.* (1993) and Teasdale *et al.* (1992, 1994) suggest that for the GalT this may be a major retention mechanism in certain cell types since increases in transmembrane domain length seem to significantly impact on GalT Golgi retention. In contrast, Burke *et al.* (1994) showed that replacement of the GlcNAcTI transmembrane domain with the larger membrane spanning domain of the TfR increases cell surface expression, but only by twofold, suggesting that other sequences and other mechanisms of retention are involved. Nilsson *et al.* (1996) saw little alteration in the HeLa cell Golgi retention of GlcNAcTI proteins with increasing lengths of polyleucine sequences replacing their transmembrane regions, also suggesting that retention mechanisms other than that proposed by the bilayer thickness model were being utilized by the GlcNAcTI. Finally, while increasing the length of the transmembrane domain in a ST-lysozyme chimera progressively increases surface expression (Munro, 1995b), increasing the length of the transmembrane domain of the intact ST protein shows no such increase in surface expression even in highly expressing Cos-1 cells (Dahdal and Colley, 1993). Since the effects of altering the length of the ST membrane region are negated when this enzyme's full stem and catalytic regions are present, this suggests that these luminal regions contribute to another process that is able to maintain this enzyme's retention in the Golgi. Comparison of the current data suggests that both the ST and GlcNAcTI have at least two separate regions that are sufficient for retention and potentially two mechanisms for retention which can act independently and/or additively depending on the cell type and level of expression.

Is Golgi retention achieved by more than one mechanism?

Neither the bilayer thickness model nor the oligomerization/kin recognition model alone can explain all of the localization results presented above. So while both models take into account differing microenvironments in individual Golgi cisternae and can explain differential localization of enzymes in various cell types, neither hypothesis alone is completely satisfying as the sole Golgi retention mechanism. While the bilayer thickness model easily accommodates the lack of sequence homology in the transmembrane domains of Golgi glycosylation enzymes, it cannot, however, explain a demonstrated requirement for sequences outside these proteins' transmembrane domains in their efficient Golgi retention. On the other hand, changes in the length of transmembrane domains, by either insertion of amino acids or complete replacement with longer sequences, have led to increased cell surface localization for wild type and chimeric proteins (Munro, 1991, 1995b; Swift and Machamer, 1991; Masibay *et al.*, 1993; Teasdale *et al.*, 1994). While these insertions could have simply increased transmembrane length, they also could have altered the presentation of specific transmembrane sequences or residues flanking the membrane spanning region and thus altered an oligomerization process. With these alternative possibilities taken into account, evidence does suggest that the length of a Golgi protein's transmembrane domain can play a role in its retention; however, the magnitude of this role will vary from protein to protein.

The oligomerization/kin recognition model predicts that formation of large oligomers prevents entry of Golgi glycosylation enzymes into transport vesicles destined for later compartments and the cell surface. While the glycosyltransferase sequences required for Golgi retention generally favor this mechanism of retention, interactions between enzymes within the same cisternae have only been observed for the medial Golgi enzymes GlcNAcTI and MannII and no large oligomers of any Golgi enzyme have been directly isolated by classical techniques. In addition, the formation of very large hetero-oligomers would presumably limit access of integral membrane protein substrates to enzymes in the center of the oligomer leading to a significant degree of inefficiency in the glycosylation process. However, in an oligomerization model based on the formation of smaller homo- or hetero-oligomers it is difficult to imagine what its preventing these smaller units

from entering transport vesicles. Cytoplasmic tethering would be attractive in this case, but there is currently no evidence for this mechanism except within the medial Golgi. Possibly, the formation of insoluble oligomers as observed for a coronavirus M glycoprotein-VSV G chimera (Weisz *et al.*, 1993), the p63 protein (Schweizer *et al.*, 1994), and the rat liver Golgi ST (Ma and Colley, unpublished results) suggests that smaller oligomers may be able to physically interact with a lipid bilayer of specific lipid composition leading to the formation of insoluble microdomains within the bilayer and retention. So while much of the available data suggest that oligomerization/kin recognition mechanism may be used for the retention of many Golgi proteins, its role in the retention of most Golgi enzymes is only preliminary. For further proof that this type of retention mechanism is used throughout the Golgi, several independent pieces of evidence suggesting the oligomerization of each enzyme or group of enzymes will be required.

While oligomerization/kin recognition could happen spontaneously and rapidly under certain environmental conditions, one obvious way to merge both models is to assume that the oligomerization/aggregation of glycosylation enzymes requires a certain threshold protein concentration before it will occur. One way of achieving this concentration may be to slow the transit of these enzymes through the Golgi by limiting their access to carrier vesicles destined for the next compartment. In this way an initial concentration step would rely on an enzyme entering a specific microenvironment, while the oligomerization event may or may not be as sensitive to environmental conditions. The concentration of a Golgi protein could be achieved by the mechanism proposed in the bilayer thickness model. As the cholesterol concentration and lipid composition gradually changes, enzymes with shorter transmembrane domains are excluded from carrier vesicles to a greater and greater extent throughout the Golgi stack. As a result the concentration of these enzymes gradually increases, and if the conditions are optimal, oligomers are formed and permanent retention results. This idea would presume that the transmembrane domain would be the primary retention region and by altering its length one should subvert the initial step in this putative two step process. As has been described above, changing the transmembrane domain length has sometimes no effect (ST; Dahdal and Colley, 1993) or only a partial effect (GlcNAcTI, Burke *et al.*, 1994; GalT, Teasdale *et al.*, 1994) on the localization of the intact protein, suggesting that alternative mechanisms of concentration may be occurring. One possibility, as described above, is that movement between the Golgi cisternae and from the TGN to the plasma membrane may require some sort of signal or level of competence (Low *et al.*, 1994, 1995). If sequences outside the transmembrane domain make these enzymes incompetent or only partially competent for forward movement in the pathway, the resulting increase in concentration could also lead to oligomerization and full retention. A type of transport incompetence could occur if these enzymes are able to interact or dock with preformed oligomers. If both the length of the transmembrane domain plus the characteristics of other sequences slow or stop the protein's transport through the secretory pathway, then retention will result, perhaps even without oligomerization.

How would this combined model explain the retention of each glycosyltransferase? I would predict that the GalT transmembrane domain would provide both concentration and oligomerization capacity, although other sequences might strengthen the interactions within the putative oligomers. On the other hand, the GlcNAcTI transmembrane and luminal sequences may be primarily involved in the concentration mechanism (whether by transmembrane domain length restraints and/or transport incompetence of another sort such as docking to preexisting complexes), with the stem sequences playing a major role and other sequences playing minor roles to direct oligomerization with the MannI, GlcNAcTII, or other resident proteins. The ST may have two independently acting modes of concentration, one based on the length of the transmembrane domain and a second related to the stem region and potentially the protein's docking to preexisting complexes. These combined may lead to complete retention with or without oligomerization depending on the cell type of expression. For each enzyme, the efficiency of one or both of these putative retention steps would be expected to rely on the cisternal microenvironment, and as that varies in different cell types, one would expect changes in localization throughout the Golgi stack and even escape from the Golgi and appearance at the cell surface.

Future directions

While it is true that we have made great strides in understanding the signals and mechanisms involved in the Golgi retention of glycosyltransferases, so much still has to be clarified, particularly with respect to mechanisms involved in this process and the functional significance of enzyme compartmentation. Some important questions that remain to be answered are (1) Why do the requirements for the Golgi retention of a specific glycosyltransferase differ in different cell types? (2) Do enzymes use multiple retention mechanisms? (3) Why are glycosylation enzymes localized differently in different cell types? and (4) What is the functional significance of these differences in glycosylation enzyme intra-Golgi compartmentation?

Why do the requirements for the Golgi retention of a specific glycosyltransferase differ in different cell types?

It has been demonstrated that the sequence requirements for the Golgi retention of a specific enzyme frequently differ in different cell types. For example, in MDCK cells, the ST transmembrane domain is sufficient for Golgi retention (Wong *et al.*, 1992), whereas in Cos cells and CHO cells the transmembrane domain plus flanking sequences are required (Munro, 1991; Dahdal and Colley, 1993; Tang *et al.*, 1995). Likewise, all three domains of the GlcNAcTI are required for efficient Golgi retention in murine L cells (Burke *et al.*, 1994), while altering the transmembrane domain in the presence of the stem region does not significantly change Golgi retention of this enzyme in HeLa cells (Nilsson *et al.*, 1996). There is also some evidence that different enzyme domains can act as independent retention regions. To understand why Golgi retention requirements vary from cell to cell, we must further investigate the roles of luminal and cytoplasmic sequences in Golgi retention in different cell types, how multiple protein domains work together to effect retention, and whether the retention of each enzyme involves multiple retention mechanisms.

Do enzymes use multiple retention mechanisms, and what are these mechanisms?

While current data for a few enzymes supports both the bilayer thickness model and the oligomerization/kin recognition model, the widespread use of these mechanisms in general

Golgi retention has not been demonstrated. We must also obtain more definitive proof that the glycosylation enzymes and other Golgi proteins are retained based on their transmembrane domain length and/or their ability to oligomerize. Transmembrane length has been shown to potentially play a role in the Golgi retention of the GalT and the ST, although the possibility that changes in the length of this region influenced other retention mechanisms (like oligomerization) cannot be ruled out (Masibay *et al.*, 1993; Munro, 1995b). Oligomerization has not been directly demonstrated for any glycosylation enzyme or set of enzymes and has only been indirectly demonstrated by ER co-retention (Nilsson *et al.*, 1994; Munro 1995b), co-immunoprecipitation (Burke *et al.*, 1994), and insolubility assays (Weisz *et al.*, 1993; Schweizer *et al.*, 1994) for a few Golgi proteins. In addition, cytoskeleton involvement in Golgi retention has only been suggested by a few indirect experiments (Weisz *et al.*, 1993; Slusarewicz *et al.*, 1994; Yamaguchi and Fukuda, 1995). A more thorough investigation of retention mechanisms and their use by the different glycosyltransferases in different cell types may tell us whether retention of a single enzyme involves one or more retention mechanism and whether certain mechanisms are more or less effective in different cell types leading to a requirement for multiple retention mechanisms in some cells. For example, it may be that in HeLa cells the GlcNAcTI is completely retained by a kin recognition via interactions in its stem, while in murine L cells the cytoplasmic and transmembrane sequences are also required either to stabilize this oligomerization process, or for another interaction (lipid bilayer or cytoskeleton) that is required for complete retention.

Why are some glycosylation enzymes localized differently in different cell types?

Differential localization of the same glycosylation enzyme in different cell types has suggested that the nature of the Golgi membranes themselves may differ from cell to cell. Presumably, differences in the lipid and cholesterol composition of membranes, cisternal pH and ion concentrations, and even associated cytoskeletal proteins, may make a difference in where a protein's transport is significantly slowed and where it is ultimately retained. The ST is localized in the trans Golgi and trans Golgi network in intestinal goblet cells, but is found throughout the Golgi in adjacent absorptive cells (Roth *et al.*, 1986). One might predict that the Golgi cisternae in these two cell types would differ in the variables mentioned above, thus leading to the different distributions of this enzyme in these cells. For example, if we use the bilayer thickness model as a mechanism of transport incompetence and retention, the cholesterol content of the early Golgi membranes may be much higher in the adsorptive cells than in the goblet cells, leading to a slower rate of transport for the ST through these early cisternae in the absorptive cells. The ST would then be detected in the cis, medial, and trans Golgi cisternae in the absorptive cells, but not until the later cisternae in the goblet cells. In the goblet cells, the ST is found in the late Golgi and at the cell surface, while in hepatocytes it is confined to the late Golgi. If we evoke an oligomerization based Golgi retention model in this case, the conditions in the late Golgi cisternae of hepatocytes may be more conducive to oligomerization and thus retention than in the intestinal goblet cells, leading to a leakage of the ST to the cell surface in the latter cell type. These two mechanisms can also easily be combined to explain the differences in goblet cell and absorptive cell ST Golgi localization and cell surface expression. Currently, we are missing data on how the Golgi cisternal microenvironments differ in different cell types and whether glycosylation enzyme oligomerization is microenvironment-specific. Understanding this could explain the observed differential localization of enzymes and tell us whether our models of retention are reasonable.

What is the functional significance of these differences in intra-Golgi compartmentation?

No data exists to tell us whether differences in intra-Golgi distribution of glycosylation enzymes makes any impact on the types of oligosaccharides expressed by cells. One might expect that the most efficient glycosylation system would strictly compartmentalize enzyme, substrate and sugar nucleotide donor or at least generally group a few enzymes in sequential reactions rather than combining all the terminal glycosylation enzymes, their substrates and donors together in one compartment. We are also missing data on the compartmentation of the specific transporters for each enzyme's sugar nucleotide donor. If these are compartmentalized in exactly the same fashion as the glycosyltransferase they serve, and sequential reactions are generally ordered throughout the Golgi stack, then we may see no discernible difference in the general carbohydrate structures synthesized by one cell relative to another cell where enzyme and transporter distribution is different. However, where we may see more of an impact is if enzymes that compete for the same substrate and make two distinct carbohydrate structures are compartmentalized differently in different cells. This may be particularly important in the terminal sialylation and fucosylation of glycoprotein and glycolipid oligosaccharides, and in the addition of sialic acid versus sulfate on penultimate GalNAc residues of glycoprotein hormone asparagine-linked oligosaccharides (Manzella *et al.*, 1996; Trinchera and Ghidoni, 1989). Essentially, it is important to understand whether an enzyme present in a particular Golgi cisternae is actually functional in that compartment or whether it is lacking appropriate substrates and donors. Recent *in vitro* techniques developed by Freeze and colleagues (Etchison and Freeze, 1996) may be the first step in determining in which intra-Golgi compartments specific enzymes are actually active.

The continuing analysis of Golgi retention signals and mechanisms at times appears quite futile to investigators in the field due to the limitations of working with mutant and chimeric proteins and the variations in cell expression systems. While results of these experiments are at times inconclusive and contradictory, we have identified general trends and should not dismiss seemingly contradictory data as unimportant. This data may be revealing processes that were not initially noticed or taken into consideration as experiments were designed. For now, there still remain more questions than answers, and more work to do before we can really state that we understand the process of Golgi enzyme retention and its role in the glycosylation of proteins and lipids.

Acknowledgments

Many thanks to Drs. Carlos Hirschberg and Jacques Baenziger and the members of my laboratory for their careful and critical reviews of the manuscript. I also give special thanks to Dr. Paul Gleeson for sharing his insights on the Golgi retention of the GalT and GlcNAcTI and to Dr. Graham Warren for providing me with his thoughts and preliminary data concerning GlcNAcTI retention.

Abbreviations

αhCG, human chorionic gonadatropin α subunit; CHO, Chinese hamster ovary; D4, dipeptidyl peptidase IV; ER, endoplasmic reticulum; FACS, fluorescence activated cell sorting; GalT, β1,4-galactosyltransferase; GlcNAcTI, N-acetylglucosaminyltransferase I; MDCK, Madin Darby canine kidney; ST, α2,6-sialyltransferase; TfR, transferrin receptor.

References

Aoki,D., Lee,N., Yamaguchi, N., Dubois,C. and Fukuda,M.N. (1992) Golgi retention of a trans-Golgi membrane protein, galactosyltransferase, requires cysteine and histidine residues within the membrane anchoring domain. *Proc Natl. Acad. Sci. USA*, **89**, 4319–4323.

Balch,W.E., McCaffery,J.M., Plutner,H. and Farquhar,M.G. (1994) Vesicular stomatitis virus glycoprotein is sorted and concentrated during export from the endoplasmic reticulum. *Cell*, **76**, 841–852.

Bartholomew,B.A., Jourdian,G.W. and Roseman,S. (1973) The sialic acids. XV. Transfer of sialic acid to glycoproteins by a sialyltransferase from colostrum. *J. Biol. Chem.*, **248**, 5751–5762.

Berger,E.G. and Hesford,F.J. (1985) Localization of galactosyl- and sialyltransferase by immunofluorescence: Evidence for different sites. *Proc. Natl. Acad. Sci. USA*, **82**, 4736–4739.

Berger,E.G., Mandel,T. and Schilt,U. (1981) Immunohistochemical localization of galactosyltransferase in human fibroblasts and HeLa cells. *J. Histochem. Cytochem.*, **29**, 364–370.

Berger,E.G., Burger,P., Hille,A., and Bachi,T. (1995) Comparative localization of mannose-6-phosphate receptor with 2, 6 sialyltransferase in HepG2 cells: an analysis by confocal double immunofluorescence microscopy. *Eur. J. Cell Biol.*, **67**, 106–111.

Bergeron,J.J.M., Paiement,J., Khan,M.N. and Smith,C.E. (1985) Terminal glycosylation in rat hepatic Golgi fractions: heterogeneous locations for sialic acid and galactose acceptors and their transferases. *Biochim. Biophys. Acta.*, **821**, 393–403.

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

Burke,J., Pettitt,J.M., Schachter,H., Sarkar,M. and Gleeson,P.A. (1992) The transmembrane and flanking sequences of β1, 2-N-acetylglucosaminyltransferase I specify *medial*-Golgi localization. *J. Biol. Chem.*, **267**, 24433–24440.

Burke,J., Pettit,J.M., Humphris,D. and Gleeson,P.A. (1994) *Medial*-Golgi retention of N-acetylglucosaminyltransferase I: contribution from all domains of the enzyme. *J. Biol. Chem.*, **269**, 12049–12059.

Chapman,R.E. and Munro,S. (1994) Retrieval of TGN proteins from the cell surface requires endosomal acidification. *EMBO J.*, **13**, 2305–2312.

Cheng,A., Le,T., Palacios,M., Bookbinder,L.H., Wassarman,P.M., *et al.* (1994) Sperm–egg recognition in the mouse: characterization of sp56, a sperm protein having specific affinity for ZP3. *J. Cell Biol.*, **125**, 867–878.

Cluett,E.B. and Brown,W.J. (1992) Adhesion of Golgi cisternae by proteinaceous interactions: intercisternal ridges as putative adhesive structures. *J. Cell Sci.*, **103**, 773–784.

Colley,K.J., Lee,E.U., Adler,B., Browne,J.K. and Paulson,J.C. (1989) Conversion of a Golgi apparatus sialyltransferase to a secretory protein by replacement of the NH_2-terminal signal anchor with a signal peptide. *J. Biol. Chem.*, **264**, 17619–17622.

Colley,K.J., Lee,E.U. and Paulson,J.C. (1992) The signal anchor and stem regions of the β-galactoside α2, 6-sialyltransferase may each act to localize the enzyme to the Golgi apparatus. *J. Biol. Chem.*, **267**, 7784–7793.

Dahdal,R.Y. and Colley,K.J. (1993) Specific sequences in the signal anchor of the β-galactoside α-2, 6-sialyltransferase are not essential for Golgi localization. *J. Biol. Chem.*, **268**, 26310–26319.

Dunphy,W.G., Brande,R. and Rothman,J.E. (1985) Attachment of terminal N-acetylglucosamine to asparagine-linked oligosaccharides occurs in central cisternae of the Golgi stack. *Cell*, **40**, 463–472.

Etchison,J.R. and Freeze,H.H. (1996) A new approach to mapping colocalization of multiple glycosyl transferases in functional Golgi preparations. *Glycobiology*, **6**, 177–189.

Evans,S.C., Lopez,L.C. and Shur,B.D. 1993. Dominant negative mutation in cell surface β1, 4-galactosyltransferase inhibits cell–cell and cell matrix interactions. *J. Cell Biol.*, **120**, 1045–1057.

Evans,S.C., Youakim,A. and Shur,B.D. (1995) Biological consequences of targeting β1, 4-galactosyltransferase to two different subcellular compartments. *BioEssays*, **17**, 261–268.

Fleischer,B., McIntyre,J.O. and Kempner,E.S. (1993) Target sizes of galactosyltransferase, sialyltransferase, and uridine diphosphatase in Golgi apparatus of rat liver. *Biochemistry*, **32**, 2076–2081.

Geuze,H.J., Slot,J.W., Strous,G.J., Hasilik,A. and Von Figura,K. (1985) Possible pathways for lysosomal enzyme delivery. *J. Cell Biol.*, **101**, 2253–2262.

Gleeson,P.A., Teasdale,R.D. and Burke,J. (1994) Targeting of proteins to the Golgi apparatus. *Glycoconjugate J.*, **11**, 381–394.

Gong,X.H., Dubois,D.H., Miller,D.J. and Shur,B.D. (1995) Activation of a G protein by aggregation of β1, 4-galactosyltransferase on the surface of sperm. *Science*, **269**, 1718–1721.

Hirschberg,C.B. (1996) Transporters of nucleotides and derivatives in the endoplasmic reticulum and Golgi apparatus. In Clapham, D.E. and Ehrlich, B.E. (eds), *Organellar Ion Channels and Transporters*. Society of General Physiologists 49th Annual Symp. Rockefeller University Press, New York, pp. 105–120.

Hoe,M.H., Slusarewicz,P., Misteli,T., Watson,R. and Warren,G. (1995) Evidence for recycling of the resident medial/trans Golgi enzyme, N-acetylglucosaminyltransferase I, in ldlD cells. *J. Biol. Chem.*, **270**, 25057–25063.

Jamieson,J.C., McCaffrey,G. and Harder,P.G. (1993) Sialyltransferase: a novel acute phase reactant. *Comp. Biochem. Physiol.*, **105**, 29–33.

Kornfeld,R. and Kornfeld,S. (1985) Assembly of asparagine-linked oligosaccharides. *Ann. Rev. Biochem.*, **54**, 631–664.

Levine,Y.K. and Wilkins,M.H.F. (1971) Structure of oriented lipid bilayers. *Nature New Biol.*, **230**, 69–72.

Leyton,L., and Saling,P. (1989) 95kD sperm proteins bind ZP3 and serve as tyrosine kinase substrates in response to zona binding. *Cell* **57**, 1123–1130.

Lopez,L.C., Youakim,A., Evans,S.C. and Shur,B.D. (1991) Evidence for a molecular distinction between Golgi and cell surface forms of β1, 4-galactosyltransferase. *J. Biol. Chem.*, **266**, 15984–15991.

Low,S.H., Tang,B.L., Wong,S.H. and Hong,W. (1994) Golgi retardation in Madin-Darby canine kidney and Chinese hamster ovary cells of a transmembrane chimera of two surface proteins. *J. Biol. Chem.*, **269**, 1985–1994.

Low,S.H., Tang,B.L., Wong,S.H. and Hong,W. (1995) Retardation of a surface protein chimera at the cis Golgi. *Biochemistry*, **34**, 5618–5626.

Ma,J. and 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.

Machamer,C.E. (1991) Golgi retention signals: do membranes hold the key? *Trends Cell Biol.*, **1**, 141–144.

Machamer,C.E. (1993) Targeting and retention of Golgi membrane proteins. *Curr. Opin. Cell Biol.*, **5**, 606–612.

Machamer,C.E. and Rose,J.K. (1987) A specific transmembrane domain of a coronavirus El glycoprotein is required for its retention in the Golgi region. *J Cell Biol.*, **105**, 1205–1214.

Machamer,C.E., Grim,M.G., Esquela,A., Chung,S.W., Rolls,M. *et al.* (1993) Retention of a cis Golgi protein requires residues on one face of a predicted alpha-helix in the transmembrane domain. *Mol. Biol. Cell.*, **4**, 695–704.

Manzella,S.M., Hooper,L.V. and Baenziger,J.U. (1996) Oligosaccharides containing β1, 4-linked N-acetylgalactosamine, a paradigm for protein-specific glycosylation. *J. Biol. Chem.*, **271**, 12117–12120.

Masibay,A.S., Balaji,P.V., Boeggeman,E.E. and Qasba,P.K. (1993) Mutational analysis of the Golgi retention signal of bovine β-1, 4-galactosyltransferase. *J. Biol. Chem.*, **268**, 9908–9916.

Munro,S. (1991) Sequences within and adjacent to the transmembrane segment of α-2, 6-sialyltransferase specify Golgi retention. *EMBO J.*, **10**, 3577–3588.

Munro,S. (1995a) A comparison of the transmembrane domains of Golgi and plasma membrane proteins. *Biochem. Soc. Trans.*, **23**, 527–530.

Munro,S. (1995b) An investigation of the role of transmembrane domains in Golgi protein retention. *EMBO J.*, **14**, 4695–4704.

Nezil,F.A. and Bloom,M. (1992) Combined influence of cholesterol and synthetic amphiphillic peptides upon bilayer thickness in model membranes. *Biophys. J.*, **61**, 1176–1183.

Nilsson,T., Lucocq,J.M., Mackay,D. and Warren,G. 1991. The membrane spanning domain of β-1, 4-galactosyltransferase specifies trans Golgi localization. *EMBO J.*, **10**, 3567–3575.

Nilsson,T., Pyaret,M., Hoe,M.H., Slusarewicz,P., Berger,E.G. and Warren,G. (1993a) Overlapping distribution of two glycosyltransferases in the Golgi apparatus of HeLa cells. *J. Cell Biol.*, **120**, 5–13.

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

Nilsson,T., Hoe,M.H., Slusarewicz,P., Rabouille,C., Watson,R., *et al.* (1994) Kin recognition between medial Golgi enzymes in HeLa cells. *EMBO J.*, **13**, 562–574.

Nilsson,T., Rabouille,C., Hui,N., Watson,R. and 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.

Orci,L., Montesano,R., Meda,P., Malaisse-Lagae,F., Brown,D. *et al.* (1981) Heterogenous distribution of filipin-cholesterol complexes across the cisternae of the Golgi. *Proc. Natl. Acad. Sci. USA*, **78**, 293–297.

Orci,L., Glick,B.S. and Rothman,J.E. (1986) A new type of coated vesicular carrier that appears not to contain clathrin: its possible role in protein transport within the Golgi stack. *Cell*, **46**, 171–184.

Paulson,J.C. and Colley, K.J. (1989) Glycosyltransferases: structure, localization, and control of cell type-specific glycosylation. *J. Biol. Chem.*, **264**, 17615–17618.

Paulson,J.C., Beranek, W.E. and Hill,R.L. (1977) Purification of a sialyltransferase from bovine colostrum by affinity chromatography on CDP-agarose. *J. Biol. Chem.*, **252**, 2356–2362.

Pelham,H.R.B. and Munro,S. (1993) Sorting of membrane proteins in the secretory pathway. *Cell*, **75**, 603–605.

Pestalozzi,D.M., Hess,M. and Berger,E.G. (1982) Immunohistochemical evidence for cell surface and Golgi localization of galactosyltransferase in human stomach, jejunum, liver and pancreas. *J. Histochem. Cytochem* , **30**, 1146–1152.

Pfeffer,S.R. and Rothman,J.E. (1987) Biosynthetic protein transport and sorting by the endoplasmic reticulum and Golgi. *Annu. Rev Biochem.*, **56**, 829–852.

Rabouille,C., Hui,N., Hunte,F., Kleckbusch,R., Berger,E.G. *et al.* (1995) Mapping the distribution of Golgi enzymes involved in the construction of complex oligosaccharides. *J. Cell Sci.*, **108**, 1617–1627.

Ratnam,S., Fraser,I.H., Collins,J.M., Lawrence,J.-A., Barrowman,J.A., and Mookerjea,S. (1981) Elevated sialyltransferase activity in the intestinal lymph of colchicine-treated rats. *Biochim Biophys Acta*, **673**, 435–442.

Roseman,S. (1970) The synthesis of complex carbohydrates by multiglycosyltransferase systems and their potential function in intercellular adhesion. *Chem. Phys. Lipids*, **5**, 270–297.

Roth,J. (1987) Subcellular organization of glycosylation in mammalian cells. *Biochim. Biophys. Acta*, **906**, 405–436.

Roth,J. and Berger,E.G. (1982) Immunocytochemical localization of galactosyltransferase in HeLa cells: codistribution with thiamine pyrophosphatase in trans-Golgi cisternae. *J. Cell Biol.*, **92**, 223–229.

Roth,J., Letze,M.J. and Berger,E.G. (1985a) Immunocytochemical demonstration of ecto-galactosyltransferase in absorptive intestinal cells. *J Cell Biol* , **100**, 118–125.

Roth,J., Taatjes,D.J., Lucocq,J.M., Weinstein,J. and Paulson,J.C. (1985b) Demonstration of an extensive trans-tubular network continuous with the Golgi apparatus stack that may function in glycosylation. *Cell*, **43**, 287–295.

Roth,J., Taatjes,D.J., Weinstein,J., Paulson,J.C., Greenwell,P. and 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.

Rothman,J.E. and Wieland,F.T. (1996) Protein sorting by transport vesicles. *Science*, **272**, 227–234.

Russo,R.N., Shaper,N.L. and Shaper,J.H. (1990) Bovine β1, 4-galactosyltransferase: two sets of mRNA transcripts encode two forms of the protein with different amino-terminal domains. *J. Biol. Chem.*, **265**, 3324–3331.

Russo,R.N., Shaper,N.L., Taatjes,D.J. and Shaper,J.H. (1992) β1, 4-Galactosyltransferase: a short NH_2-terminal fragment that includes the cytoplasmic and transmembrane domain is sufficient for Golgi retention. *J Biol. Chem.*, **267**, 9241–9247.

Schweizer,A., Rohrer,J., Hauri,H.-P. and Kornfeld,S. (1994) Retention of p63 in an ER-Golgi intermediate compartment depends on the presence of all three of its domains and on its ability to form oligomers. *J. Cell Biol.*, **126**, 25–39.

Shaper,J.H. and Shaper,N.L. (1992) Enzymes associated with glycosylation. *Curr. Opin. Struct. Biol.* **2**, 701–709.

Shaper,N.L., Mann,P.L. and Shaper,J.H. (1985) Cell surface galactosyltransferase: immunochemical localization. *J. Cell. Biochem.*, **28**, 229–239.

Shur,B.D. (1993) Glycosyltransferases as cell adhesion molecules. *Curr. Opin. Cell Biol.*, **5**, 854–863.

Slusarewicz,P., Nilsson,T., Hui,N., Watson,R. and Warren,G. (1994) Isolation of a matrix that binds medial Golgi enzymes. *J. Cell Biol.*, **124**, 405–413.

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

Swift,A.M., and Machamer,C.E. (1991) A Golgi retention signal in a membrane-spanning domain of coronavirus El protein. *J. Cell Biol.*, **115**, 19–30.

Taatjes,D.J., Roth,J., Shaper,N.L. and Shaper,J.H. (1992) Immunocytochemical localization of β1, 4-galactosyltransferase in epithelial cells from bovine tissues using monoclonal antibodies. *Glycobiology*, **2**, 579–589.

Taatjes,D.J., Roth,J., Weinstein,J. and Paulson,J.C. (1988) Post-Golgi apparatus localization and regional expression of rat intestinal sialyltransferase detected by immunoelectron microscopy with polypeptide epitope-purified antibody. *J. Biol. Chem.*, **263**, 6302–6309.

Taatjes,D.J., Roth,J., Weinstein,J., Paulson,J.C., Shaper,N.L. and Shaper,J.H. (1987) Codistribution of galactosyl- and sialyltransferase: reorganization of trans Golgi apparatus elements in hepatocytes in intact liver and cell culture. *Eur. J Cell Biol.*, **44**, 187–194.

Tang,B.L., Low,S.H., Wong,S.H. and Hong,W. (1995) Cell type differences in Golgi retention signals for transmembrane proteins. *Eur. J. Cell Biol.*, **66**, 365–374.

Tang,B.L., Wong,S.H., Low,S.H. and Hong,W. (1992) The transmembrane domain of N-glucosaminyltransferase I contains a Golgi retention signal. *J. Biol. Chem.*, **267**, 10122–10126.

Teasdale,R.D., D'Agostaro,G. and Gleeson,P.A. (1992) The signal for Golgi retention of bovine β1, 4-galactosyltransferase is in the transmembrane domain. *J. Biol. Chem.*, **267**, 4084–4096.

Teasdale,R.D., Matheson,F. and Gleeson,P.A. (1994) Post-translational modifications distinguish cell surface from Golgi-retained β1, 4 galactosyltransferase molecules. Golgi localization involves active retention. *Glycobiology*, **4**, 917–928.

Trinchera,M. and Ghidoni,R. (1989) Two glycosphingolipid sialyltransferases are localized in different sub-Golgi compartments in rat liver. *J. Biol. Chem.*, **264**, 15766–15769.

Tulsiani,D.R.P., Opheim,D.J. and Touster,O. (1977) Purification and characterization of α-D-mannosidase from rat liver Golgi membranes. *J. Biol. Chem.*, **252**, 3227–3233.

Velasco,A., Hendricks,L., Moreman,K.W., Tulsiani,D.R.P., Touster,O. and Farquhar,M.G. (1993) Cell type-dependent variations in the subcellular distribution of α-mannosidase I and II. *J Cell Biol.*, **122**, 39–51.

Wahlberg,J.M., Geffen,I., Reymond,F., Simmen,T. and Spiess,M. (1995) *trans*-Golgi retention of a plasma membrane protein: mutations in the cytoplasmic domain of the asialoglycoprotein receptor subunit H1 result in *trans*-Golgi retention. *J. Cell Biol.*, **130**, 285–297.

Walter,P. and Johnson,A.E. (1994) Signal sequence recognition and protein targeting to the endoplasmic reticulum membrane. *Annu. Rev. Cell Biol.*, **10**, 87–119.

Weisz,O.A., Swift,A.M. and Machamer,C.E. (1993) Oligomerization of a membrane protein correlates with its retention in the Golgi complex. *J Cell Biol.*, **122**, 1185–1196.

Wieland,F.T., Gleason,M.L., Serafini,T.A. and Rothman,J.E. 1987. The rate of bulk flow from the endoplasmic reticulum to the cell surface. *Cell*, **50**, 289–300.

Wong,S.H., Low,S.H. and Hong,W. (1992) The 17-residue transmembrane domain of β-galactoside α2, 6-sialyltransferase is sufficient for Golgi retention. *J. Cell Biol.*, **117**, 245–258.

Yamaguchi,N. and Fukuda,M.N. (1995) Golgi retention mechanism of β-1, 4-galactosyltransferase: membrane-spanning domain-dependent homodimerization and association with α- and β-tubulins. *J. Biol. Chem.*, **270**, 12170–12176.

Youakim,A., Dubois,D.H. and Shur,B.D. 1994. Localization of the long form of β-1, 4-galactosyltransferase to the plasma membrane and Golgi complex of 3T3 and F9 cells by immunofluorescence confocal microscopy. *Proc. Natl. Acad. Sci. USA*, **91**, 10913–10917.

Zambrano,F., Fleischer,S. and Fleischer,B. (1975) Lipid composition of the Golgi apparatus of rat kidney and liver in comparison with other subcellular organelles. *Biochim Biophys. Acta*, **380**, 357–369.

Received May 5, 1996, accepted on June 20, 1996.