Nucleotide sugar transporters of the Golgi apparatus

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Introduction

The Golgi apparatus is the major site of protein, lipid and proteoglycan glycosylation. The glycosylation enzymes, as well as kinases and sulfatases that catalyze phosphorylation and sulfation, are localized within the Golgi cisternae in characteristic distributions that frequently reflect their order in a particular pathway (Kornfeld and Kornfeld 1985; Colley 1997). The glycosyltransferases, sulfotransferases and kinases are "transferases" that require activated donor molecules for the reactions they catalyze. For eukaryotic, fungal and protozoan glycosyltransferases these are the nucleotide sugars UDP-*N*-acetylglucosamine (UDP-GlcNAc), UDP-galactose (UDP-Gal), GDP-fucose (GDP-Fuc), CMP-sialic acid (CMP-Sia), UDP-glucuronic acid (UDP-GlcA), GDP-mannose (GDP-Man), and UDP-xylose (UDP-Xyl) (Hirschberg et al. 1998). For the kinases, ATP functions as the donor, while for the sulfotransferases, adenosine 3′-phosphate 5′-phosphate (PAPS) acts as the donor (Hirschberg et al. 1998). The active sites of all these enzymes are oriented towards the lumen of the Golgi cisternae. This necessitates the translocation of their donors from the cytosol into the lumenal Golgi compartments. In this chapter we will focus on the structure, function and localization of the Golgi nucleotide sugar transporters (NSTs), and highlight the diseases and developmental defects associated with defective transporters. We direct the reader to several excellent reviews on Golgi transporters for additional details and references (Hirschberg et al. 1998; Berninsone and Hirschberg 2000; Gerardy-Schahn et al. 2001; Handford et al. 2006; Caffaro and Hirschberg 2006).

The identification of NSTs and the diseases and defects in development caused by mutant transporters

Abundant evidence now exists for the importance of glycoconjugates in both development and in fundamental processes in adult organisms (Varki 1993; Haltiwanger and Lowe 2004). The critical role of NSTs and the maintenance of nucleotide sugar levels in the glycosylation of proteins, lipids and proteoglycans has been highlighted by a number of transporter mutants that lead to developmental defects in model organisms such as *C. elegans* and *Drosophila*, to decreased virulence of parasites such as *Leishmania*, and to severe diseases in humans and cattle.

In early studies, mutants exhibiting altered glycosylation in both mammalian cell lines and other organisms, such as yeast and the protozoan parasite *Leishmania*, were isolated and found defective in nucleotide sugar transport by biochemical analysis (Ballou et al. 1991; Descoteaux et al. 1995; Herman and Horvitz 1999; Patnaik and Stanley 2006). In these analyses, investigators quantified the transport of radiolabeled sugar nucleotides into sealed vesicles under different conditions using filtration or centrifugation to separate the vesicles from the assay medium (reviewed in Hirschberg et al. 1998). For example, Deutscher et al. (1984) demonstrated that Lec2 CHO cells possessed only 2% of the CMP-Sia transport activity of wild type CHO cells, while others demonstrated similar decreases in UDP-GlcNAc transport activity in the *Kluyveromyces lactis mnn2-2* mutant (Abeijon et al. 1996a), in UDP-Gal transport activity in the MDCKII-RCA^r mutant (Brandli et al. 1988), and in GDP-Man transport activity in both the *L. donovani* C3PO mutant and in the *Saccharomyces cerevisiae vrg4* mutant (Ma et al. 1997; Dean et al. 1997). These studies demonstrated that nucleotide sugar transport was absolutely required for glycosylation in mammalian, yeast and protozoan cells and provided investigators with a way to clone the defective transporters by complementation. The cloning of NST coding sequences opened the way to further characterization of the structure and function of the transporters by expression in heterologous systems and reconstitution into proteoliposomes. This also allowed investigators to identify inactivating NST mutations leading to developmental defects and human disease.

Defects in the GDP-fucose and CMP-Sia transporters lead to two congenital disorders of glycosylation

Leukocyte adhesion deficiency syndrome type II (LAD II), also called congenital disorder of glycosylation (CDG) IIc, is a rare autosomal recessive human syndrome characterized by a general reduction of fucose in glycoconjugates due to a deficiency in GDP-Fuc transport (reviewed in Hirschberg 2001; Becker and Lowe 1999). Patients have an abnormal facial appearance and exhibit severe psychomotor and growth retardation, recurrent infections, and periodontitis. Using patient fibroblasts and screening for recovery of glycoconjugate fucosylation in transformants, both Lübke et al. (2001) and Lühn et al. (2001) cloned the human and *C. elegans* GDP-fucose transporters, respectively. These investigators and Helmus et al. (2006) have identified several specific mutations in LADII/CDG IIc patients that lead to disease.

The murine CMP-Sia transporter was cloned by complementation of sialylation deficient Lec2 CHO cells and its activity verified by heterologous expression in *S. cerevisiae* (Eckhardt et al. 1996; Berninsone et al. 1997). Recently, inactivating mutations in this transporter were found to result in a new CDG type II that was diagnosed in a 4-month-old boy (Willig et al. 2001; Martinez-Duncker et al. 2005). Decreased sialylation in the patient led to macrothrombocytopenia, neutropenia, and complete lack of the sialyl Lewis X antigen on polymorphonuclear cells. The patient experienced progressive

hemorrhaging, respiratory distress syndrome, and opportunistic infections. Ultimately, complications including pulmonary viral infection, massive pulmonary hemorrhage, and respiratory failure led to death at the age of 37 months (Willig et al. 2001).

Drosophila Fringe connection and *C. elegans* SQV7, two multi-substrate NSTs required for signaling and cell interactions during development

The *Drosophila* Fringe connection transporter was cloned by Selva et al. (2001) and Goto et al. (2001) who identified mutants in Fringe connection in screens for segment polarity and limb defects. These mutants had defects in Fringe-dependent Notch signaling, and in the Wingless/Wnt, Hedgehog, and fibroblast growth factor signaling pathways that require heparan sulfate expression. Fringe is a GlcNAc transferase that modifies O-linked fucose residues on the Notch receptor's epidermal growth factor repeats, and this modification differentially modulates the binding of Notch to receptors and its signaling pathways (Haltiwanger and Lowe 2004). Accordingly, Fringe connection is a Golgi localized multi-substrate nucleotide sugar transporter that transports UDP-GlcNAc, UDP-GlcA, and UDP-Xyl (Selva et al. 2001). Other groups cloned putative human orthologs of Fringe connection (Suda et al. 2004; Ishida et al. 2005), and over expression of one of these proteins in mammalian cells increased surface levels of heparan sulfate, consistent with the activity of *Drosophila* Fringe connection (Suda et al. 2004).

C. elegans sqv mutants exhibit a squashed vulval phenotype and a reduction in hermaphrodite fertility (Herman et al. 1999). All eight of these mutant genes encode proteins involved in different aspects of proteoglycan biosynthesis (Herman and Horvitz 1999; Hwang and Horvitz 2002). The *sqv7* gene encodes an NST that transports UDP-GlcA, UDP-GlcNAc, and UDP-Gal in a competitive and non-cooperative fashion (Berninsone et al. 2001). Surprisingly, two other *C. elegans* NSTs, the SRF-3 and CO3H5.2 proteins, are redundant with SQV-7 and each other, and they exhibit a dramatically different non-competitive and simultaneous mechanism. The implications of this will be discussed below.

A defective UDP-GlcNAc transporter leads to complex vertebral malformation in cattle

The yeast and canine UDP-GlcNAc transporters were cloned by complementation of the transporter defect the *K. lactis mnn2-2* mutant (Abeijon et al. 1996b; Guillen et al. 1998). Interestingly, the sequence similarity of these two functionally equivalent transporters from different species is very low (22%), but not uncommon among transporters with the same specificity from different species. This and the high sequence similarities observed between transporters with different specificities, highlights the importance of biochemically verifying the true substrates of recombinant NSTs (Caffaro and Hirschberg 2006). Recently, a recessively inherited disease in cattle, complex vertebral malformation, was found to be the result of a missense mutation in

the bovine UDP-GlcNAc transporter (Thomsen et al. 2006). The disease, which results in severe malformation of the vertebrae, abortion of fetuses, and perinatal death, has been reported in cattle all over the world. In fact, it was reported that approximately 30% of the elite sires in Japan and Denmark are carriers for this disease (Thomsen et al. 2006).

The GDP-mannose transporter is required for the virulence of parasites and is an essential protein in yeast

Protozoans and yeast differ from vertebrates in that they require the translocation of GDP-Man into the Golgi lumen for extensive mannosylation of their glycoconjugates. GDP-Man transporters were cloned by complementation of the *S. cerevisiae vrg4* mutant and the *L. donvani* C3PO mutant (Descoteaux et al. 1995; Ma et al. 1997; Poster and Dean 1996). The importance of this transporter in both organisms is underscored by the fact that *vrg4* is an essential gene in yeast, and by the requirement for GDP-Man transport and the biosynthesis of mannose-containing surface glycoconjugates for *Leishmania* virulence. The latter observation identifies the *Leishmania* LPG2 GDP-Man transporter as a possible drug target for the treatment of Leishmaniasis.

Nucleotide sugar transporter specificity and mechanism

In early studies, biochemical assays employing topologically correct membrane vesicles were used to identify and characterize NST activities. These initial studies showed that transport in most cases is organelle specific, is temperature sensitive, saturable (K_ms of 1–10 μM), concentrates nucleotide sugars 50- to 100-fold in Golgi vesicles/liposomes relative to their concentration in the assay medium, does not require ATP, is not altered by ionophores, and that NSTs are antiporters (Hirschberg et al. 1998). These initial observations were verified by expression of recombinant NSTs in heterologous systems and the reconstitution of purified, recombinant transporters into proteoliposomes. Importantly, these more recent studies demonstrated that single transporter proteins were sufficient for transport activity and revealed surprising multi-substrate specificities and unique mechanisms for some NSTs.

Antiporter mechanism

The ability of NSTs to function as antiporters, where the nucleotide sugar is stoichiometrically exchanged for the corresponding nucleoside monophosphate (see Fig. 1), was supported by early studies which showed that preloading nucleoside monophosphates into Golgi membrane vesicles, or proteoliposomes containing transporters, stimulated the transport of their respective nucleotide sugars into the lumen of these vesicles (Hirschberg et al. 1998). More recently both the recombinant *L. donovani* LPG2 GDP-Man transporter and the murine CMP-Sia transporter were reconstituted into
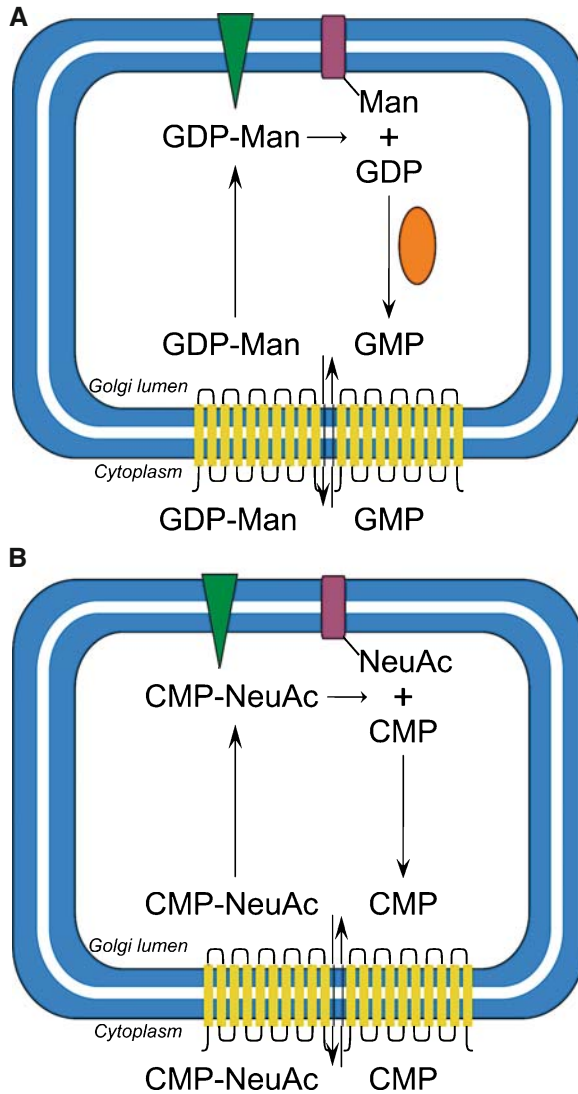
Figure 1. The nucleotide sugar transport/antiport cycle in the Golgi apparatus. (**A**) GDP-Man, which is synthesized in the cytoplasm, is transported into the Golgi lumen by the GDP-Man transporter. In the Golgi lumen, GDP-Man is a substrate for mannosyltransferases (triangle), which transfer Man to glycoconjugate substrates (rectangle). GDP, the other product of the transfer reaction, is converted by a lumenal nucleoside diphosphatase (oval) to GMP. The export of GMP to the cytosol is coupled to the import of GDP-Man. (**B**) This type of antiport mechanism occurs for the exchange of CMP-Sia and CMP using a distinct CMP-Sia transporter. The major difference is that no diphosphatase activity is needed because CMP is released following the sialyltransferase reaction.

phosphatidylcholine liposomes and their specificity and mechanism reevaluated (Segawa et al. 2005; Tiralongo et al. 2006). In both cases investigators observed that preloading the liposomes with the corresponding nucleotide monophosphate yielded a 3-fold higher initial rate of transport relative to liposomes that were not preloaded, again supporting an antiporter mechanism. Other work by Tiralongo et al. (2006) using the reconstituted murine CMP-Sia transporter showed that the rate of CMP-Sia transport was also stimulated under "equilibrium exchange conditions" and suggested that this transporter is a simple mobile carrier with a binding site that alternates between both sides of the membrane.

Most biochemical evidence suggests that the nucleotide monophosphate is exchanged for the corresponding nucleotide sugar (reviewed in Hirschberg et al. 1998). This requires that the nucleoside diphosphates released after transfer of the sugar to the glycoconjugate substrate are converted to nucleoside monophosphates by diphosphatases (Fig. 1A). Of course, CMP-Sia is the one exception because CMP is directly released following sugar transfer (Fig. 1B). Additional evidence for the importance of disphosphatase activities in the nucleotide sugar antiporter mechanism came from the finding that deletion of the *S. cerevisiae* Golgi guanosine diphosphatase (Gda1) decreased GDP-Man transport into membrane vesicles and led to a partial defect in the addition of Man to both glycoproteins and glycolipids (Abeijon et al. 1993; Berninsone et al. 1994). Other work by D'Alessio et al. (2005) demonstrated that GDP-Man dependent glycosylation is reduced but not eliminated in nucleoside diphosphatase mutants in yeast, and suggested that other mechanisms may lead to nucleoside monophosphate translocation. Recent studies by Muraoka et al. (2007) in which the transporter mechanism was evaluated for the endoplasmic reticulum (ER) localized human UGTrel7 transporter, that is capable of transporting UDP-Gal, UDP-GlcA and UDP-GlcNAc, and the Golgi localized *Drosophila* Fringe connection transporter, suggested that the former transporter may be a UDP-sugar/UDP-sugar antiporter, while the latter may use UDP as efficiently as UMP as an antiport substrate.

Multi-specificity and redundancy of NSTs

Several NSTs have been identified as multi-substrate transporters including the *C. elegans* SQV-7 transporter and the *Drosophila* Fringe connection transporter described above. Other multi-substrate and redundant NSTs emerged as investigators searched the human, *Drosophila* and yeast genomes for putative nucleotide sugar transporters, and subjected these newly cloned putative transporters to extensive analyses using multiple nucleotide sugars (Segawa et al. 2002; Muraoka et al. 2001; Ashikov et al. 2005). For example, the UDP-Gal transporter was first cloned by complementation of mammalian and yeast mutants (Miura et al. 1996; Tabuchi et al. 1997). Later, Segawa et al. (2002) cloned the Drosophila UDP-Gal transporter and found that both the human UDP-Gal transporter 1 (hUGT1) and the *Drosophila* transporter were

specific for both UDP-Gal and UDP-GalNAc. More recently, Capul et al. (2007) identified two genes (LPG5A and LPG5B) in *Leishmania major* that encode UDP-Gal transporters with partially overlapping activity. The multi-specificity and redundancy of NSTs raised new questions concerning mechanism and potential differences in the roles of NSTs with similar substrate specificities. Investigators have now begun to address some of these questions.

The *C. elegans* genome encodes 18 putative NSTs based on sequence homology with transporters from other species, but the transport of only seven nucleotide sugars is required for glycosylation, and a similar situation exists in humans (Caffaro et al. 2007; Martinez-Duncker et al. 2003). Three redundant, multi-substrate specific *C. elegans* NSTs, SQV7, SRF-3, and CO3H5.2, have been characterized. As described above, the SQV-7 protein is a multi-substrate transporter that is specific for UDP-GlcA, UDP-GlcNAc, and UDP-Gal and transports these substrates in a competitive and non-cooperative fashion (Berninsone et al. 2001). In contrast to the *sqv* mutants, the *C. elegans srf* mutants exhibit no obvious behavioral or morphological changes, but do have defects in cell surface molecules that alter their binding to antibodies and lectins and block infection/colonization by parasites (reviewed in Hoflich et al. 2004). The *srf-3* gene encodes a transporter that is specific for both UDP-Gal and UDP-GlcNAc (Hoflich et al. 2004), while the CO3H5.2 gene encodes a transporter specific for UDP-GlcNAc and UDP-GalNAc (Caffaro et al. 2006). Surprisingly, the CO3H5.2 and SRF-3 transporters use a simultaneous and non-competitive substrate transport mechanism that differs from the competitive mechanism used by the SQV7 transporter (Caffaro et al. 2006, 2007). A deletion of 16 amino acids in the loop between transmembrane (TM) helices 2 and 3 of the CO3H5.2 protein preferentially decreased UDP-GalNAc transport by 85–90%, but did not impact UDP-GlcNAc transport, suggesting two independent translocation sites for these nucleotide sugars (Caffaro et al. 2006).

The existence of these three *C. elegans* transporters that exhibit partially overlapping substrate specificity (UDP-GlcNAc) and expression patterns, led Caffaro et al. (2007) to investigate this redundancy. They used RNAi technology to knock down the CO3H5.2 gene in *srf-3* mutants and found that a defect in both transporters led to developmental and morphological changes not observed in the *srf-3* mutant alone. This strongly suggested that these two transporters are at least partially redundant and begged the question why redundancy was needed. One possibility, suggested by the investigators, is that certain nucleotide sugars need to be maintained at high levels so that processes requiring these molecules can proceed with high efficiency under a variety of circumstances. In addition, if nucleotide sugar levels drop, different glycosylation pathways can be differentially affected depending upon the affinity of the associated glycosyltransferases for the particular nucleotide sugar. For example, in the MDCKII-RCA^r cell mutant where availability of UDP-Gal is limited, a decreased galactosylation is observed for glycoproteins, glycolipids and keratan sulfate proteoglycans,

while the amounts of chondroitin and heparan sulfate proteoglycans remain relatively normal (Toma et al. 1996). Similarly, in LADII/CDG IIc patients who have a defect in the GDP-Fuc transporter, low dose oral Fuc supplementation therapy partially restores P-selectin-mediated, but not E-selectin-mediated binding of neutrophils (Marquardt et al. 1999a, b). Since the synthesis of P- and E-selectin glycan ligands depends upon different Fuc transferase activities (Huang et al. 2000), it is likely that the enzymes have different affinities for GDP-Fuc and that this leads to differences in glycoconjugate expression under limiting GDP-Fuc levels. Another possible reason that there may be genetic pressure to maintain the expression of rendundant multi-substrate transporters is so that the non-overlapping functions of these transporters can be maintained (Caffaro et al. 2006). A third possibility, is that different transporters with similar specificity function in conjunction with specific glycosylation pathways. This idea is supported by the work of Capul et al. (2007) who cloned two UDP-Gal transporters from *L. major* (LPG5A and LPG5B) and demonstrated that deficiencies in these transporters impacted the biosynthesis of the two predominant *Leishmania* surface glycoconjugates differently.

NST structure and sequence requirements for function, trafficking, and localization

Topology and oligomerization
Hydrophobicity plots and topology prediction algorithms based on the deduced amino acid sequences of NSTs suggest that these proteins are multi-spanning membrane proteins containing six to ten TM regions with both their amino- and carboxy-termini in the cytosol (Hirschberg et al. 1998) (Fig. 2). Initial studies to define the topology of the *K. lactis* UDP-GlcNAc transporter in Golgi vesicles have been performed and suggest either a six or eight TM helix topology (Berninsone and Hirschberg 2000). In contrast, Eckhardt et al. (1999) evaluated the membrane topology of the murine CMP-Sia transporter using immunofluorescence microscopy following epitope insertion and selective membrane permeablization, and obtained data supporting a 10 TM helix model for this transporter (Eckhardt et al. 1999). Further studies are needed to define the topological arrangement of other NSTs.

Most NSTs are thought to exist as homodimers. For example, the rat liver UDP-GalNAc and GDP-Fuc transporters both migrate with molecular masses of approximately 40 kDa upon denaturing gel electrophoresis, but exhibit molecular masses of 80–90 kDa in native glycerol gradients (Puglielli et al. 1999; Puglielli and Hirschberg 1999). VRG4, the *S. cerevisiae* GDP-Man transporter has also been found to be a homodimer (Gao and Dean 2000). A carboxy-terminal region of this protein, which includes the last TM helix, is necessary for dimer formation, and truncated proteins lacking this sequence are unstable and rapidly degraded. Interestingly, overexpression of an amino-terminal truncated VRG4 protein in yeast causes a dominant negative growth
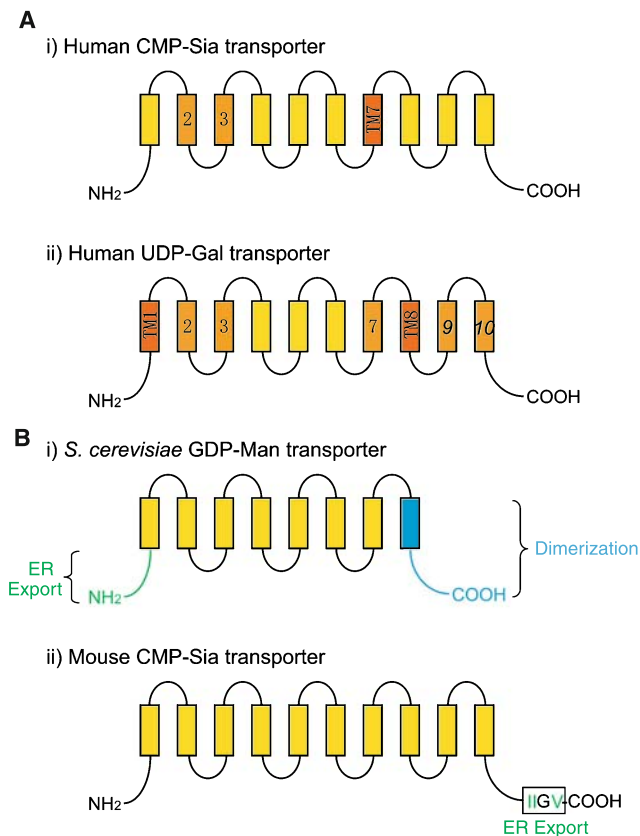
Figure 2. Topology and functional regions of the Golgi NSTs. (**A**) Topology and TM helices required for transport activity. (i) CMP-Sia transporter: TM helix 7 (TM7) is required for the specificity of this transporter for CMP-Sia, whereas the TM helices 2 and 3 enhance the efficiency of CMP-Sia transport. (ii) UDP-Gal transporter: TM helices 1 (TM1) and 8 (TM8) are necessary but not sufficient for UDP-Gal transport, and other helices in different combinations (2, 3 and 7 OR 9 and 10) must be included with TM1 and TM8 for transport activity. (**B**) Sequences required for dimerization and ER export. (i) In the GDP-mannose transporter, a carboxy-terminal sequence containing the last TM helix is involved in dimerization, whereas the amino-terminal 44 amino acids include an ER export signal. (ii) In the CMP-Sia transporter, a di-isoleucine motif and a terminal valine residue (boxed) at the very carboxy-terminus mediate its ER export.

phenotype. This is believed to be a consequence of the formation of inactive heterodimers of the truncated protein and the endogenous full length protein, and supports the notion that homodimerization of VRG4 is crucial for its function. In contrast LPG2, the *Leishmania* GDP-Man transporter, which migrates as a hexamer in native glycerol gradients and upon pore-limited native gel electrophoresis (Hong et al. 2000). Further functional studies will be needed to determine whether the hexamer form of this protein is the active state in the membrane.

Sequence and structural requirements for substrate recognition and transport

The identification of mutants in NSTs first gave hints to what regions and amino acid residues are critical for their function. Gao et al. (2001) identified a conserved region in the yeast Vrg4 GDP-Man transporter (amino acids 280–291) as the transporter's GDP-Man binding site, because mutations in this sequence reduced binding to a photoaffinity substrate analog and led to decreased GDP-Man transport. Gerardy-Schahn and colleagues (Eckhardt et al. 1998; Oelmann et al. 2001) identified the various mutations in the Lec2 and Lec8 complementation groups that lead to defects in the CMP-Sia transporter and UDP-Gal transporter, respectively. While many of the identified mutants of the CMP-Sia transporter were deletions that led to mislocalization and low expression, the Gly189Glu mutant was localized in the Golgi and well-expressed, suggesting that this amino acid was critical for transporter activity per se (Eckhardt et al. 1998). Likewise, the ΔSer213 and Gly281Asp mutants of the UDP-Gal transporter were localized properly and expressed well, but still inactive. Interestingly, introducing these changes into the CMP-Sia transporter also led to its inactivation, suggesting that these conserved residues are important for general transporter mechanism (Oelmann et al. 2001).

Aoki et al. (2001, 2003) identified critical TM helices in both the CMP-Sia and UDP-Gal transporters. These transporters are 43% identical and yet are absolutely specific for their respective substrates. The investigators created chimeras containing TM helices from both transporters and found that CMP-Sia transporter TM helix 7 was necessary and sufficient for transport of CMP-Sia when inserted into a UDP-Gal transporter background, while the inclusion of TM helices 2 and 3 enhanced efficiency of transport. In contrast, TM helices 1 and 8 of the UDP-Gal transporter were necessary but not sufficient for UDP-Gal transport in the context of the CMP-Sia transporter. Only the inclusion of either TM helices 9 and 10, or TM helices 2, 3 and 7 from the UDP-Gal transporter, in addition to TM helix 1 and 8, could generate a chimeric transporter competent to transport UDP-Gal (Aoki et al. 2003).

Sequence requirements for NST ER export and retrieval

In eukaryotic secretory pathway, exit of secretory proteins from the ER relies on their sorting into ER-derived COPII-coated vesicles. Much of this sorting is mediated by specific, cytoplasmically exposed signals that can be recognized by subunits of the COPII coat (Barlowe 2003). ER export signals have been found in several NSTs. The amino-terminal 44 amino acids of VRG4, the yeast Golgi GDP-Man transporter, are likely to include an ER export signal because deletion of this region leads to ER accumulation, and fusion of these sequences to related ER proteins promotes their transport to the Golgi apparatus (Gao and Dean 2000). A di-isoleucine motif and a terminal valine in the last four amino acids of the carboxy-terminal cytoplasmic tail of the murine CMP-Sia transporter mediate the ER export of the protein (Zhao et al.

2006). These signals are independent and both need to be deleted or replaced to abolish ER export.

Signals predicted to allow the COPI-coated vesicle-mediated ER retrieval have been found in both ER NSTs and some Golgi NSTs (Martinez-Duncker et al. 2003). The cloning of the gene for a second functional isoform of the human UDP-Gal transporter (hUGT2) predicted that this protein is truncated at its carboxy-terminus (Ishida et al. 1996). Comparison of the localization of the two isoforms revealed that hUGT1 is localized in the Golgi, while hUGT2 is localized in the ER and Golgi (Kabuß et al. 2005). Kabuß et al. (2005) demonstrated that a dilysine motif (LysValLysGlySer) found in the carboxy-terminal cytoplasmic tail of hUGT2 was responsible for its ER retrieval and its dual localization. Fusion of this motif is sufficient to redistribute the Golgi CMP-Sia transporter to the ER. A similar dilysine ER retrieval motif also is found in the *K. lactis* Golgi UDP-GlcNAc transporter, however it is unclear whether this motif actually functions as an ER retrieval signal (Abeijon et al. 1996b). Interestingly, work done by Abe et al. (2004) suggests that a COPI-mediated retrieval of the GDP-Man transporter to the ER is a critical step in the Golgi localization of this transporter and that lysine residues in its carboxyl-terminal cytoplasmic tail are necessary for COPI coat interaction and retrieval.

Golgi targeting of NSTs and the organization of glycosylation machinery

Many studies on the signals and mechanisms of Golgi glycosylation enzyme localization have revealed that sequences mediating Golgi localization are complex and may reside in different domains of these type II membrane proteins (Colley 1997). Likewise, several redundant mechanisms including those involving lipid partitioning, oligomerization, and retrieval, may be used to maintain Golgi enzymes in their resident cisternae (Colley 1997; Mironov et al. 2005). When considering the organization of glycosylation pathways in the Golgi, it is tempting to speculate that NSTs are co-compartmentalized with the glycosyltransferases that use their nucleotide sugar substrates as donors and that they may even form functional complexes. For this reason we were surprised to find that the CMP-Sia transporter showed a more restricted *medial–trans* Golgi localization than might be indicated by the rather broad Golgi distribution of sialyltransferases involved in both the sialylation of glycoproteins and glycolipids (Zhao et al. 2006). This finding, together with others (D'Alessio et al. 2003; Kabuß et al. 2005), suggests that CMP-Sia as well as other nucleotide sugars move freely in the lumen of the Golgi apparatus. This is consistent with recent evidence that the Golgi cisternae are more interconnected than once believed (Mironov et al. 2005).

The identity of the sequences that mediate the Golgi localization of the NSTs has not been widely investigated, however the sequence requirements for the Golgi localization of some viral multi-spanning membrane proteins have been determined. The first TM helix of the avian coronavirus E1 protein is sufficient to localize two cell surface proteins to the Golgi and is likely to

play a role in mediating E1 Golgi localization (Machamer and Rose 1987). Later studies showed that uncharged polar residues that line one face of this TM helix are important for Golgi localization (Swift and Machamer 1991). Work by Locker et al. (1994) suggested that the cytoplasmic tail of the mouse hepatitis virus M protein plays a role in its Golgi localization. We have recently found that, while the CMP-Sia transporter cytoplasmic sequences have no direct role in Golgi localization, fusing the first TM helix of the transporter to the lumenal sequences of an inefficiently retained Golgi sialyltransferase can reduce the level of its Golgi exit (W. Zhao and K. J. Colley, unpublished data). This suggests that, like the Golgi localization of the infectious bronchitis virus E1 protein, the first TM helix of the CMP-Sia transporter may be involved in its Golgi localization.

NST Golgi localization could also be mediated by interactions with glyco-syltransferases. Complex formation between a transporter and its corresponding glycosyltransferase would presumably enhance the efficiency of the glycosylation reaction by facilitating the transfer of the nucleotide sugar to the glycosyltransferase. Along these lines, Sprong et al. (2003) found that a portion of the Golgi UDP-Gal transporter can associate with the ER localized UDP-galactose:ceramide galactosyltransferase to allow UDP-Gal import into this compartment. However, evidence for other functional NST-glycosyltransferase complexes is lacking. Radiation inactivation studies suggest that the galactosyl- and sialyltransferases are not in functional complexes with the corresponding transporters (Fleischer et al. 1993). Moreover, redistribution studies show that there is no complex formation between the CMP-Sia transporter and the corresponding sialyltransferases (Zhao et al. 2006). Although weak interactions between glycosyltransferases and their respective NSTs are still possible, these results suggest that NSTs may not rely on glycosyltransferases for their Golgi localization.

Conclusions

Investigators have made great progress in identifying NSTs and defining their substrate specificity. However, many questions remain concerning the mechanism of nucleotide sugar transport, the roles and expression of multi-substrate and redundant NSTs, the potential connection of redundant transporters with specific glycosylation pathways, the mechanisms of NST Golgi localization, and how NSTs and glycosyltransferases are organized within the Golgi apparatus. For example, how is the developmental, cellular and tissue expression of redundant and multi-substrate NSTs controlled? Do differences in sub-Golgi localization of NSTs with similar substrate specificity allow these transporters to be compartmentalized with glycosylation enzymes in different pathways? How does the simultaneous transport of two substrates occur in one transporter? What are the precise interactions mediating nucleotide sugar recognition and transport? How are NSTs localized in the Golgi, and how are they organized vis a vis their respective glycosyltransferases to

promote efficient glycosylation? The realization that mutant NSTs lead to human disease and developmental defects has and will continue to generate interest in these proteins and will hopefully stimulate additional research to answer these many questions.

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