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Nucleotide Sugar Transporters of the Golgi Apparatus: From Basic Science to Diseases

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ABSTRACT
Approximately 80% of secreted and membrane proteins (40% of all proteins) of eukaryotes become covalently linked to sugars in the lumen of the Golgi apparatus, a cellular organelle that is part of the secretory system of all eukaryotes. The sugar donors are mostly nucleoside diphosphate sugars (nucleotide sugars) and must be translocated from the cytosol, their site of synthesis, across the Golgi apparatus membrane and into the lumen by specific transporters. These are hydrophobic, homodimeric proteins that span the membrane multiple times. Mutants of these proteins have developmental phenotypes including diseases in humans and cattle.

Transporters of Nucleotide Sugars across the Golgi Apparatus Membrane Are Required for Protein Glycosylation in the Golgi Apparatus Lumen

In eukaryotes, half of cellular proteins are secreted or membrane bound. Both groups of proteins are synthesized on membrane bound ribosomes, translocated into the lumen of the endoplasmic reticulum, and transported via vesicles to the Golgi apparatus and from there to their final destination within or outside the cell. In the lumen of the endoplasmic reticulum and Golgi apparatus, 80% of these proteins undergo covalent addition of sugars (glycosylation). These may be added to the proteins directly or to other sugars, which in turn are covalently linked to proteins. The sugar donors are nucleotide sugars (Figure 1), which transfer their sugars in enzyme catalyzed reactions to their acceptors. In some instances, ATP[46] and PAPS[46] (Figure 1) are substrates for covalent additions of phosphate and sulfate to proteins and sugars in the lumen of the Golgi apparatus. All these nucleotide derivatives must be transported from the cytosol, where most are synthesized, across the Golgi apparatus membrane into the lumen of this organelle by specific transporter proteins.

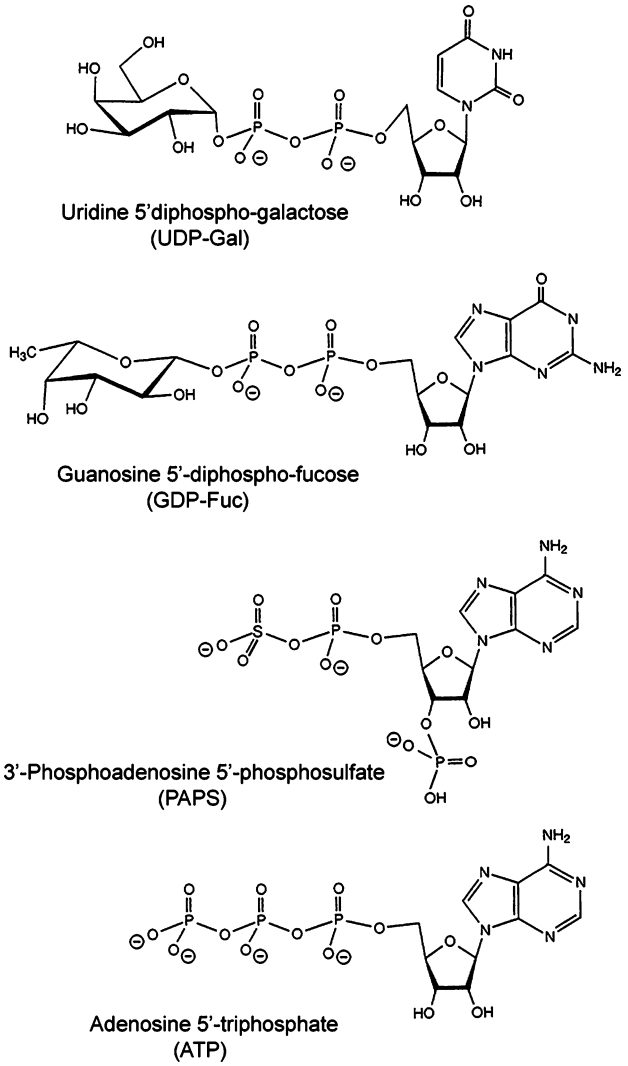

FIGURE 1. Structures of nucleotide sugars, ATP, and PAPS. Glucose is the C-4 epimer of galactose. Mannose is the C-2 epimer of glucose.

General Biochemical Characteristics of Golgi Apparatus Nucleotide Sugar Transport

Before several of these transporters were cloned, their transport activities were characterized using a combination of biochemical and cell biological approaches. These consisted of using highly purified Golgi apparatus vesicles, usually from rat liver, which were sealed and were in the same membrane orientation as *in vivo*.[1] These vesicles

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Carlos B. Hirschberg was born in Santiago, Chile, in 1943. He attended the University of Chile where he obtained the degree of Biochemist *cum laude* and the University of Illinois—Urbana where he received the Ph.D. in Chemistry. Following postdoctoral training at Harvard Medical School and the Massachusetts Institute of Technology, he held faculty positions at the biochemistry departments at St. Louis University School of Medicine and the University of Massachusetts Medical School. In 1998, he became Professor and founding Chair of the Department of Molecular and Cell Biology at Boston University Goldman School of Dental Medicine. An NIH-NIGMS MERIT awardee, his laboratory co-workers discovered the transport/antiport system for nucleotide derivatives in the Golgi apparatus and were the first to purify and clone proteoglycan sulfotransferases.

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were incubated separately with radioactive solutes that were either standard membrane penetrators or non-penetrators. Following centrifugation, the amount of each solute in the vesicle pellet was measured. The volumes accessible to these solutes in the vesicle pellets were then calculated from the specific activities. The volumes accessible to radioactive nucleotide sugars were also determined and compared to the volume of the standards. Typically, accessible volumes for nucleotide sugars were found to be larger than those for standard membrane penetrators, suggesting that transport of these nucleotide sugars is protein mediated. Incubations *in vitro* of these vesicles with nucleotide sugars labeled with different radioisotopes in the purine or pyrimidine base and the sugars demonstrated that (a) the entire nucleotide sugar was transported,[1] (b) transport was saturable with K_m values in the range of 1−10 μM,[1] (c) some nucleotide sugars were transported solely across the Golgi apparatus membrane while others were also transported across the membrane of the endoplasmic reticulum,[1] and (d) nucleotide sugars and their components became concentrated many fold in the lumen of Golgi apparatus vesicles relative to their concentration in the reaction medium.[1] The energy for transport was not provided by ATP but by the *coupled equimolar* exit of the corresponding nucleoside monophosphate[1−5] (Figure 2). This is called an antiport system, analogous to the ATP−ADP exchanger of mitochondria and chloride−bicarbonate exchanger of red blood cells.

The rat liver Golgi apparatus transporters for UDP-GalNAc,[13,46] GDP-fucose,[8] ATP,[14] and PAPS[15] appear to be active in the membrane as homodimers. The same conclusion was obtained for the GDP-mannose transporter of *Saccharomyces cerevisiae*.[16] While the transporter for GDP-mannose of *Leishmania donovani* was described as a hexamer, functional studies have not determined whether this is the active oligomer in the membrane.[17]

Previous studies with Golgi apparatus vesicles *in vitro* showed that transport of nucleotide sugars was competitively inhibited by the corresponding nucleoside mono- and diphosphates. While transport depended on the specific sugar covalently bound to the nucleoside phosphate, the specific free sugar did not inhibit transport by itself. This suggested that the nucleoside moiety was necessary for initial binding of substrate to the transporter protein and that sugars were essential for translocation specificity.[18,19] Qualitative studies have used chimeras of the human UDP-Gal[46] and CMP-sialic acid transporters, which share 43% primary sequence identity, to determine regions of each protein necessary for transport of each of the nucleotide sugars.[20,21] These studies should be a valuable tool in future crosslinking studies between substrates and transporters to determine, together with mutagenesis of the amino acids involved, the actual substrate recognition features of the individual transporter proteins.

Earlier studies with Golgi apparatus-enriched vesicles from mutant mammalian cells also showed that mutants in UDP-galactose transport activity were highly specific

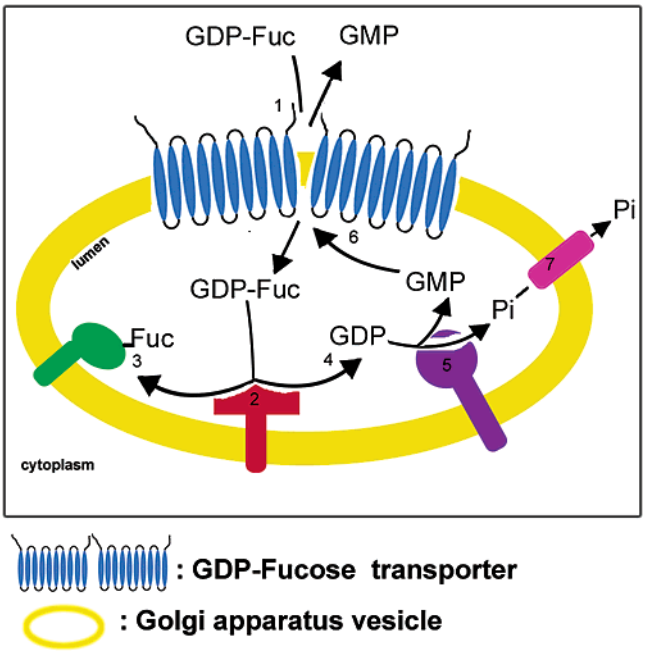

FIGURE 2. The Golgi apparatus GDP-fucose transport/antiport cycle. GDP-fucose, which is synthesized in the cytosol, is transported intact into the Golgi lumen (**1**),[6] where it is a substrate for fucosyltransferases (**2**), enzymes that transfer fucose to proteins (**3**). Guanosine diphosphate (GDP), the other reaction product (**4**), is converted by a luminal nucleoside diphosphatase (**5**) to guanosine monophosphate and inorganic phosphate. GMP leaves the Golgi apparatus lumen in a coupled exchange with GDP-fucose (**6**). Phosphate probably exits via a phosphate transporter, GOLAC (**7**).[7] The GDP-fucose transporter appears to be a homodimer within the membrane. Its topography within the membrane, with both amino and carboxy termini facing the cytosol, is inferred from other studies of the topography of nucleotide sugar transporters.

for deficiencies in this one particular nucleotide sugar. These studies therefore demonstrated that there was not a common uridine nucleotide sugar transporter in these cells.[1] Analyses of the biochemical phenotype of these mutants showed that glycoproteins, proteoglycans, and glycolipids were deficient in the particular sugar for which the corresponding nucleotide sugar transport was deficient.[1] This demonstrated a direct relationship between transport and subsequent covalent addition of the corresponding sugar to macromolecules. Studies with the nematode *Caenorhabditis elegans*, described later, further illustrate this point.

Reconstitution of Golgi Apparatus Transporters into Proteoliposomes: An Important Tool

Native and recombinant Golgi apparatus nucleotide sugar transporters have been reconstituted into unilamellar phosphatidylcholine liposomes. This has had several purposes: (a) to determine that transport is mediated by specific proteins (several of which have been purified to apparent homogeneity) and to demonstrate that no accessory proteins are required for transport, (b) to use as assays in the purification of several transporters, (c) to obtain direct evidence that transporters function as anti-

porters with the corresponding nucleoside monophosphate, (d) to characterize kinetic components of transport activities and to determine the affinity and specificity of transporter proteins for substrates, and (e) to obtain direct evidence that mutants exhibiting a partial loss of nucleotide sugar transport activity into Golgi apparatus vesicles *in vitro* are the result of less intrinsic transport activity of the particular transporter protein *per se*. The latter rules out the possibility that the particular mutant protein is completely inactive and that activities of other transporters are responsible for the partial nucleotide sugar transport observed in Golgi vesicles of the mutant cells.

The first Golgi apparatus nucleotide transport activities to be reconstituted were those for CMP-sialic acid[22] and PAPS.[22] The procedure was subsequently used as an assay to purify to apparent homogeneity Golgi apparatus transporters for UDP-GalNAc,[13] GDP-fucose,[8] ATP,[14] and PAPS.[15]

Reconstituted proteoliposomes were also used to demonstrate that Golgi apparatus nucleotide sugar transporters function as antiporters by showing transport stimulation following preloading of liposomes with the corresponding nucleoside monophosphate but not nucleoside diphosphate.[4,5,22] Reconstitution studies also showed that the K_m value for transport of a particular nucleotide sugar obtained from proteoliposomes and Golgi apparatus vesicles were very similar, in the low micromolar range,[4,5,22] and that the substrate specificity, inferred from analyses of nucleotide sugar transport with vesicles from mammalian mutant cells, was correct. For example the purified rat liver UDP-GalNAc transporter was *not* active in transport of any other uridine containing nucleotide sugar.[13] Recently wild-type and mutant human GDP-fucose transporters with the same mutations as those found in leukocyte adhesion deficiency syndrome II (LAD II) patients were expressed in bacteria. Following solubilization with detergents and reconstitution into liposomes, transport activities were measured. Mutants had significant transport activity strongly suggesting that the mutations are partial loss of function of the GDP-fucose transporter proteins *per se* (Figure 3). The relative transport activities *in vitro* were also consistent with the severity of the disease.[23]

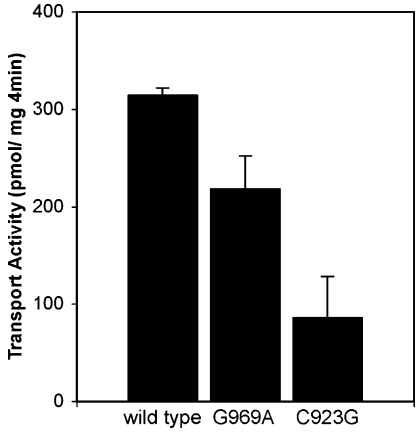

FIGURE 3. GDP-fucose transporters from humans and two different patients with leukocyte adhesion deficiency syndrome II were expressed in *E. coli*. Following solubilization with Triton-X-100 and removal of most of the detergent, proteins were reconstituted into unilamellar phosphatidylcholine liposomes. Transport of GDP-fucose was measured at a 2 μM concentration as previously described.

Heterologous Expression of Golgi Apparatus Nucleotide Sugar Transporters: Lessons Learned from Molecular Cloning

An important advance in the molecular cloning of many nucleotide sugar transporters has been the ability to express such proteins from one eukaryotic species in another[24,25] while at the same time retaining its original solute transport functionality. This was important toward allowing the use of a mammalian cDNA library to clone the mammalian transporter for UDP-GlcNAc by correction of the cell surface phenotype of a mutant of the yeast *Kluyveromyces lactis*.[25] This mutant, which had been shown to be deficient in transport of this nucleotide sugar into Golgi apparatus vesicles *in vitro*, has decreased surface binding to *Griffonia simplicifolia*, a lectin that recognizes terminal α or β linked *N*-acetylglucosamine. The decreased lectin binding enabled cloning of the yeast *K. lactis* UDP-GlcNAc transporter by measuring restoration of *Griffonia simplicifolia* binding to wild-type levels.[26]

The fact that a mammalian cDNA was able to correct the above mutant yeast phenotype also demonstrated that the Golgi apparatus targeting signals of these proteins are conserved across species, initially between mammals and yeast but later extended to other species.[11] Subsequently, functional expression of Golgi apparatus transporters from one eukaryotic species to another has been used to (a) show that putative transporters identified using a bioinformatics approach are indeed nucleotide sugar transporters, (b) identify multiple substrates for a particular nucleotide sugar transporter, and (c) obtain relatively large amounts of transporter proteins for subsequent purification and reconstitution into liposomes. Nucleotide sugar transporters from mammals, nematodes, insects, plants, and protozoa have been expressed in the yeast *S. cerevisiae*, which has the practical advantage of having only two *endogenous* nucleotide sugar transport activities, those for UDP-Glc[46] and GDP-mannose. For example, following expression in *S. cerevisiae* of a putative transporter that has been tagged to visualize its expression, a Golgi apparatus vesicle enriched fraction is isolated, and transport of different nucleotide sugars is measured *in vitro*. For transport to have occurred, the signal must be (a) temperature dependent with values at 30 °C higher than those at 0 °C, (b) several fold higher than that obtained following transformation of yeast with vector without insert, and (c) saturable with a K_m value in the 1−30 μM range. In addition, when possible, one should attempt to correct the phenotype of mutants previously characterized as being defective in a particular Golgi apparatus nucleotide sugar transport activity. When biochemical assays suggest more than one possible substrate for the transporter in question, demonstration that the substrates are competitive or not is important (see studies below with *C. elegans*).

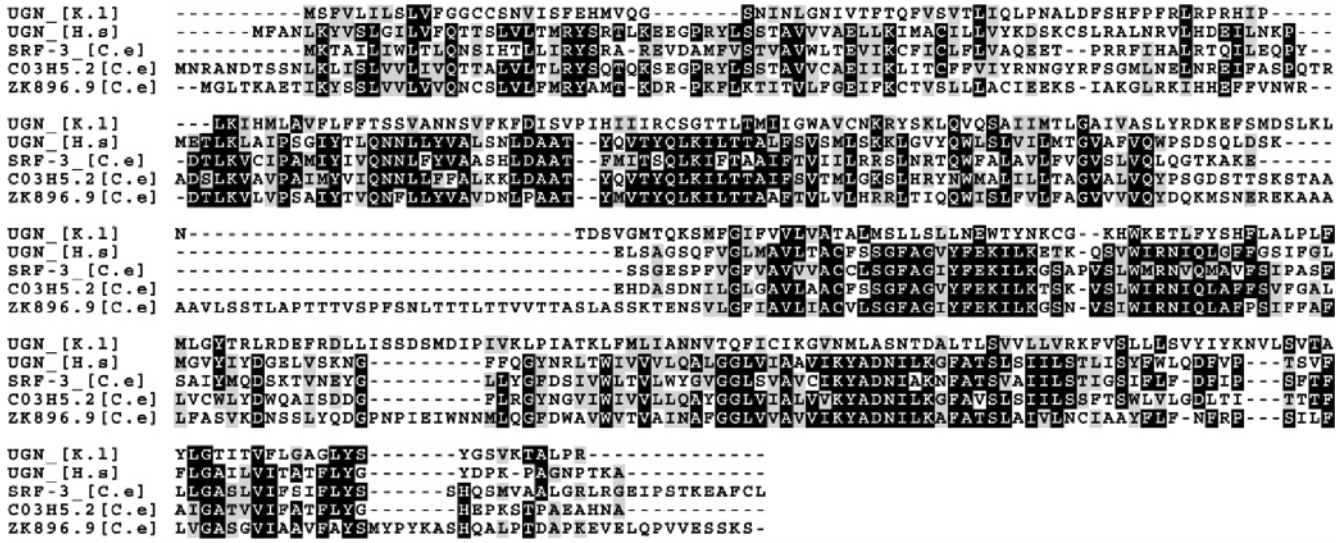

FIGURE 4. Amino acid sequence alignment of nucleotide sugar transporters CO3H5.2 (AAB66127), ZK896.9 (CAB05326), and SRF-3 (NP_502447) from *C. elegans*, UDP-*N*-acetylglucosamine (AAC49313) from *K. lactis*, and UDP-*N*-acetylglucosamine (NP_036375) from *Homo sapiens*. The Clustal W method was used. Identical amino acids are in black. Similar ones are in gray. Substrates for SRF-3 are UDP-Gal and UDP-GlcNAc, for CO3H5.2 UDP-GalNAc and UDP-GlcNAc, and for ZK896.9 UDP-Gal, UDP-GalNAc, and UDP-GlcNAc.

Molecular cloning of Golgi apparatus nucleotide sugar transporters has revealed several important features: (a) To date all of these transporters appear to be hydrophobic proteins spanning the membrane multiple times. In two instances, the murine CMP-sialic acid transporter and the yeast *K. lactis* UDP-GlcNAc transporter, *direct* evidence has been obtained for this topographic orientation. In both cases, the amino and carboxy termini appear to be cytosolic, suggesting an even number (6 or 10) of transmembrane spanning domains.[11,12] The reader is cautioned that most membrane spanning algorithms used in the literature for Golgi apparatus nucleotide sugar transporters fail to take into account that the membrane of the Golgi apparatus is approximately 20% thinner than the plasma membrane, for which most algorithms are designed. (b) Primary sequence is generally *not* an indicator of substrate specificity. Forty to fifty percent of sequence identity might result in very different substrate transport specificities such as among the transporters for CMP-sialic acid and UDP-galactose.[11,21] Other examples of this are shown in Figure 4. (c) The same substrate specificity may be mediated by proteins with as low as 22% sequence identity such as transporters for UDP-GlcNAc of canine kidney MDCK cells and the yeast *K. lactis*. The former was cloned by phenotypic correction of a mutant in the latter species.[11] Here again the reader is cautioned that annotations of substrate specificities of these transporters in bioinformatic databases may not be correct.

The Phenotypes of Mutants in the Nucleotide Sugar Transport/Antiport Cycle

Early studies with nucleotide sugar transport mutant cells grown in tissue culture showed that these cells did not have a morphological or growth phenotype even though their glycoproteins, glycosaminoglycans, and glycolipids showed drastic reductions in the particular sugar for which the nucleotide sugar transporter was deficient.[1] Similar results were found with a mutant of the yeast *K. lactis* that has reduced transport of UDP-GlcNAc.[1] However, subsequent studies with uni- and multicellular organisms have shown profound developmental and growth changes in such nucleotide sugar transporter mutants, among them *Leishmania donovani*,[27] *Drosophila melanogaster*,[28,29] *Caenorhabditis elegans*,[30−32] humans,[33,34] and more recently cattle.[35]

Mutants in the Golgi apparatus luminal nucleotide diphosphatases have biochemical phenotypes and in some instances morphological and developmental ones as well.[36,37] These are enzymes that give rise to nucleoside monophosphates, the antiporter molecules in the Golgi apparatus nucleotide sugar transport/antiport cycle. Mutations in these enzymes most likely will lead to impaired nucleotide sugar transport into the Golgi apparatus lumen as a consequence of decreased nucleoside monophosphate (the antiporter molecule) exit from the Golgi apparatus lumen. Such mutants in the yeasts *S. cerevisiae* and *Schizosaccharomyces pombe*, while having biochemical phenotypes in Golgi glycosylation, do not appear to have a biochemical phenotype in transient glucosylation of proteins occurring in the endoplasmic reticulum for reasons that are currently not known.[38]

Because Golgi apparatus nucleotide sugar transporters provide substrates for the biosynthesis of many different sugar containing macromolecules (glycans), it is only the particular function of these macromolecules in a given organism that determines a particular biochemical, morphological, and growth phenotype. Thus the yeast *S. cerevisiae VRG-4* gene, which encodes the GDP-mannose transporter, is necessary for viability.[39] In the pathogenic parasite *Leishmania donovani*, the *LPG-2* gene encodes a GDP-mannose transporter whose deficiency leads to avirulence, while mutations in the homologous gene in *Leishmania mexicana* do not.[27,40]

Table 1. *Caenorhabditis elegans* Nucleotide Sugar Transporters Comparison: Substrate Specificity And Biological Implications

	SQV-7[30,42,43,45]	SRF-3[31,44]	C03H5.2[32]
substrate specificity			
UDP-Gal	+	+	−
UDP-GlcNAc	−	+	+
UDP-GalNAc	+	−	+
UDP-glucuronic acid	+	−	−
tissue expression pattern of transporter	vulva seam cells oocytes	pharyngeal gland cells seam cells spermatheca	pharyngeal gland cells seam cells, spermatheca vulva, body wall muscle stomatointestinal muscle
morphological phenotype of mutant (when grown on *E. coli*)	squashed vulva reduced hermaphrodite fertility	wheat germ agglutinin surface (srf) binding	RNAi in wild-type animals: disorganization of body muscle fibers RNAi in srf-3 animals: oocyte accumulation abnormal gonad migration
chemical phenotype of loss of function mutants	reduced chondroitin and heparan sulfate	reduced O- and N-linked glycoconjugates	not determined

Nucleotide Sugar Transporter Mutants of *Caenorhabditis elegans*: New Concepts

The most detailed studies on nucleotide sugar transporters in a multicellular organism have been done in the nematode *C. elegans*, the first multicellular organism for which the complete genome was determined. Examination of its genome suggested that it encoded 20 nucleotide sugar transporters, while based on analyses of the sugar content of this nematode, only seven nucleotide sugars would be required. Insights into this apparent dilemma, also found in humans, have been obtained from recent studies with *C. elegans*, which have already led to major novel concepts regarding functions of nucleotide sugar transporters in multicellular organisms. Among these are the following: (a) nucleotide sugar transporters are not solely specific for one substrate, as was shown in initial studies in other organisms, but may translocate multiple substrates (Figure 4 and Table 1); (b) nucleotide sugar transporters are expressed in specific tissues of multicellular organisms (Table 1); (c) nucleotide sugar transporters show genetic enhancement.[41] Thus, a mutation in one nucleotide sugar transporter gene intensifies the phenotype caused by a mutation in another nucleotide sugar transporter gene (Table 1). For example, RNAi against one nucleotide sugar transporter in wild-type nematodes has no morphological phenotype, while RNAi against the same transporter in a mutant of another nucleotide sugar transporter results in multiple morphological phenotypes. In the above example, the transporters have in common at least one substrate, suggesting functional redundancy among the proteins.

SQV-7 is a *C. elegans* nucleotide sugar transporter identified in a screen of nematodes with a squashed vulva (SQV) phenotype.[42] The particular gene encodes a putative protein that spans the membrane several times and has 30−40% amino acid identity to other known and characterized nucleotide sugar transporters.[42] Because we had previously shown that 50% amino acid sequence identity was insufficient to determine the substrate specificity of these transporters, heterologous expression of the *C. elegans* gene in *S. cerevisiae* led to the then surprising result that SQV-7 could transport UDP-Gal, UDP-glucuronic acid, and UDP-GalNAc.[30] Each of these transport activities was saturable with K_m values in the 1−10 μM range. Transport of UDP-Gal was inhibited by the other two nucleotide sugars in a competitive, non-cooperative manner, while UDP-GlcNAc, which was not a substrate and only differs from UDP-GalNAc by stereochemistry at carbon 4 of the sugar, did not inhibit. The two mutants in SQV-7 have a concomitant decrease in all the transport activities.[30] Finally, the UDP-Gal transport activity of SQV-7 corrected the phenotype of a mammalian mutant specifically defective in UDP-Gal transport.[30] Analyses of glycans deficient in SQV-7 mutants showed that both chondroitin and heparan sulfate were diminished by approximately one-half and that the length of chondroitin chains was shorter than wild type, while the disaccharides of heparan sulfate were not affected.[43] This is consistent with the substrate specificity of SQV-7, which predicts that chains of chondroitin, containing *N*-acetylgalactosamine, but not heparan sulfate, which contains *N*-acetylglucosamine, would be affected. Because galactose is part of the linkage region of both polymers, it is not surprising that their biosynthesis is also affected. The SQV-7 transporter was localized in the vulva, seam cells, and oocytes.[45]

SRF-3, another *C. elegans* multisubstrate nucleotide sugar transporter, was identified following a screen of mutants resulting in surface labeling with lectins to which wild-type animals are refractile. SRF-3 transports UDP-Gal and UDP-GlcNAc,[31] as determined by heterologous expression in *S. cerevisiae* and phenotypic correction of mammalian and yeast mutants defective in transport of UDP-Gal and UDP-GlcNAc, respectively. While mutants lacking SRF-3 activity do not show a morphological phenotype, when grown on *Escherichia coli*, they are resistant to infection by the bacterial pathogens *Microbacterium nematophilum* and do not bind to the biofilm of bacterial plates containing *Yersinia pseudotuberculosis*. The transporter is localized in spermatheca, glandular g1 and g2 cells, and seam cells, all of which are secretory cells. The latter are thought to play an important role in

cuticle synthesis.[31] This result is also consistent with the finding that SRF-3 mutants show a 65% reduction in acidic *O*-linked glycoconjugates containing glucuronic acid and galactose, as well as a reduction in *N*-linked glycoconjugates containing galactose and fucose.[44]

CO3H5.2 is a recently characterized nucleotide sugar transporter of *C. elegans* that has been instrumental in raising new concepts regarding transport mechanisms and interactions among these transporters in a multicellular organism. It transports UDP-GalNAc and UDP-GlcNAc based on heterologous expression in *S. cerevisiae* and phenotypic correction of a *K. lactis* mutant defective in transport of UDP-GlcNAc.[32] Unexpectedly, transport of each of the two substrates appears to be independent and not competitively inhibited as previously found for SQV-7.[30] A deletion mutant was characterized that transports only 10−15% of UDP-GalNAc while transport of UDP-GlcNAc is virtually unaffected (Caffaro, unpublished results). This is in marked contrast to two SQV-7 mutants, where transport of all three substrates was decreased to a similar extent.[32] CO3H5.2 is expressed in the same cells as SRF-3 and also in wall muscle, in intestinal muscle, and in the vulva. While RNAi of CO3H5.5 in a hypersensitive wild-type strain shows only disorganization of muscle fibers, RNAi in SRF-3 mutants causes several additional phenotypes such as oocyte accumulation and abnormal gonad migration. These results provide direct evidence for genetic enhancement among nucleotide sugar transporters. Because of the partial overlap in tissue expression and substrate specificity among CO3H5.2 and SRF-3, we hypothesize that functional redundancy among these transporters and their products may be important to ensure adequate product formation (Table 1).

Human Nucleotide Sugar Transporter Mutants: Leukocyte Adhesion Deficiency Syndrome II

Leukocyte adhesion deficiency syndrome type II is a rare autosomal recessive human syndrome characterized by a general reduction of fucose in glycoconjugates. The specific molecular defect is the result of mutations in the GDP-fucose transporter.[9,10,33] The disease was initially described in Arab children in Israel and has also been observed in children of Turkish, Pakistani, and Brazilian origin, as well as a child from a different location as the original ones in Israel. The children have severe growth and mental retardation, abnormal facial appearance, neurologic abnormalities, immunodeficiencies with recurrent bacterial infections, leukocytosis, and the unusual blood group phenotype called Bombay. This is blood group O without terminal fucose. Additional biochemical hallmarks of the disease is a lack of expression of fucosylated glycoconjugates such Lewis X, sialyl Lewis X, and fucosylated proteins. Lymphoblasts from patients with LAD II transport GDP-fucose *in vitro* into their Golgi apparatus vesicles at approximately one-third the V_{max} of normal lymphoblasts and those from parents, the latter being heterozygous for the mutation. The parents do not have any clinical symptoms of the disease. Specific examples of two mutations in the human GDP-fucose transporter described so far are shown in Figure 3. Cloning of the wild-type transporter was accomplished by using a human cDNA library to identify the specific cDNA that corrected the phenotype of LAD II fibroblasts and was orthologous to the *C. elegans* GDP-fucose transporter. Both approaches yielded cDNAs encoding a putative protein of 40 kDa.[9,10] This is in good agreement with the previously purified rat liver GDP-fucose transporter, which had a MW of 39 kDa.[8]

Drosophila melanogaster Nucleotide Sugar Transporter Mutants

Two independent studies showed that homozygous mutants in the *Drosophila* gene *fringe connection*, encoding a nucleotide sugar transporter, have profound developmentally impaired phenotypes including nicked wings and shorter legs. The substrate specificity of the *fringe connection* gene product was determined by heterologous expression in *L. donovani* and *S. cerevisiae*.

Unfortunately the results of these studies were not the same: one study suggested a transporter for UDP-glucuronic acid, UDP-GlcNAc, and UDP-xylose, while the other suggested, in addition to the previous substrates, UDP-GalNAc, UDP-Gal, and UDP-Glc.[28,29] This discrepancy is most likely a technical problem resulting from the transport assay used in each case. The latter study showed no evidence of substrate saturation, while the former did so only with one substrate. This further illustrates the importance of rigorous biochemical analyses in determining substrates for transporters using approaches outlined in a previous section.

Bovine Nucleotide Sugar Transporter Mutants: Complex Vertebral Malformation

Complex vertebral malformation is a recessively inherited disorder of bovines leading to abortion of fetuses or perinatal death and vertebral abnormalities such as fused vertebrae at the cervico-thoracic junction. The disease was first detected in 1999 and has now been found in The Netherlands, United Kingdom, United States, Japan, and Denmark, the latter two countries where up to approximately 30% of the Holstein cattle population are heterozygotic carriers. Recent studies have shown the molecular defect to be a missense mutation in the UDP-GlcNAc transporter, specifically a V180F substitution.[35] This information can be used for prenatal diagnosis and further illustrates the profound phenotypic consequences of a mutation in a single nucleotide sugar transporter in mammals, analogous to those previously discussed in human LAD II.

Conclusions

We foresee two general directions for future studies of Golgi apparatus nucleotide sugar transporters. One general direction consists of (a) biophysical studies using

crystallography and NMR, helping in the elucidation of the structure and detailed mechanism of action of these transporters in the Golgi apparatus membrane, (b) development of specific inhibitors of transport, which should cross the plasma membrane to be active on the cytosolic side of the Golgi apparatus membrane, and (c) synthesis of nucleotide derivatives with photoaffinity reactive sites to determine to which amino acids nucleotide substrates are bound. The second general direction consists of biological studies in multicellular organisms using a combination of mutants, RNAi technology, and chemical analyses of glycans. Such approaches should shed further light on the role of such transporters and the particular sugars of the nucleotide sugars in normal and pathological development processes in these organisms.

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References

(1) Hirschberg, C. B.; Robbins, P. W.; Abeijon, C. Transporters of Nucleotide Sugars, ATP and Nucleotide Sulfate in the Endoplasmic Reticulum and Golgi Apparatus. *Annu. Rev. Biochem.* **1998**, *67*, 49−69.

(2) Capasso, J. M.; Hirschberg, C. B. Mechanisms of Glycosylation and Sulfation in the Golgi Apparatus: Evidence for Nucleotide Sugar/Nucleoside Monophosphate and Nucleotide Sulfate/Nucleoside Monophosphate Antiports in the Golgi Apparatus Membrane. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 7051−7055.

(3) Waldman, B. C.; Rudnik, G. UDP-GlcNAc Transport Across the Golgi Membrane: Electroneutral Exchange for Dianionic UMP. *Biochemistry* **1990**, *29*, 44−52.

(4) Segawa, H.; Soares, R. P.; Kawakita, M.; Beverley, S. M.; Turco, S. J. Reconstitution of GDP-mannose Transport Activity with Purified *Leishmania* LPG2 Protein in Liposomes. *J. Biol. Chem.* **2005**, *280*, 2028−2035.

(5) Tiralongo, J.; Ashikov, A.; Routier, F.; Eckhardt, M.; Bakker, H.; Gerardy-Schahn, R.; von Itzstein, M. Functional Expression of the CMP-sialic Acid Transporter in *Escherichia coli* and its Identification as a Simple Mobile Carrier. *Glycobiology* **2006**, *16*, 73−81.

(6) Sommers, L. W.; Hirschberg, C. B. Transport of Sugar Nucleotides into Rat Liver Golgi. *J. Biol. Chem.* **1982**, *257*, 10811−10817.

(7) Nordeen, M. H.; Jones, S. M.; Howell, K. E.; Caldwell, J. H. GOLAC An Endogenous Anion Channel of the Golgi Complex. *Biophys. J.* **2000**, *78*, 2918−2928.

(8) Puglielli, L; Hirschberg, C. B. Reconstitution, Identification and Purification of the Rat Liver Golgi Membrane GDP-fucose Transporter. *J. Biol. Chem.* **1999**, *274*, 35596−35600.

(9) Luhn, K.; Wild, M. K.; Eckhardt, M.; Gerardy-Schahn, R.; Vestweber, D. The Gene Defective in Leukocyte Adhesion Deficiency II Encodes a Putative GDP-fucose Transporter. *Nat. Genet.* **2001**, *28*, 69−72.

(10) Lubke, T.; Marquardt, T.; Etzioni, A.; Hartmann, E.; v. Figura, K.; Korner, C. Complementation Cloning Identifies CDG-IIc, A New Type of Congenital Disorders of Glycosylation, as a GDP-fucose Transporter Deficiency. *Nat. Genet.* **2001**, *28*, 73−76.

(11) Berninsone, P. M.; Hirschberg, C. B. Nucleotide Sugar Transporters of the Golgi Apparatus. *Curr. Opin. Struct. Biol.* **2000**, *10*, 542−547.

(12) Gerardy-Schahn, R.; Oelmann, S.; Bakker, H. Nucleotide Sugar Transporters: Biological and Functional Aspects. *Biochemie* **2001**, *83*, 775−782.

(13) Puglielli, L.; Mandon, E. C.; Rancour, D. M.; Menon, A. K.; Hirschberg, C. B. Identification and Purification of the Rat Liver Golgi Membrane UDP-*N*-acetylgalactosamine Transporter. *J. Biol. Chem.* **1999**, *274*, 4474−4479.

(14) Puglielli, L.; Mandon, E. C.; Hirschberg, C. B. Identification and Purification of the Rat Liver Golgi Membrane ATP Transporter. *J. Biol. Chem.* **1999**, *274*, 12665−12669.

(15) Mandon, E. C.; Milla, M. E.; Kempner, E.; Hirschberg, C. B. Purification of the Golgi Adenosine 3′-phosphate 5′-phosphosulfate Transporter, a Homodimer within the Golgi Membrane. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 10707−10711.

(16) Gao, X.-D.; Dean, N. Distinct Domains of the Yeast Golgi GDP-mannose Transporter Mediate Oligomer Assembly and Export from the Endoplasmic Reticulum. *J. Biol. Chem.* **2000**, *275*, 17718−17727.

(17) Hong, K.; Ma, D.; Beverley, S. M.; Turco, S. J. The *Leishmania* GDP-mannose Transporter Is an Autonomous, Multispecific, Hexameric Complex of LPG2 Subunits. *Biochemistry* **2000**, *39*, 2013−2022.

(18) Capasso, J. M.; Hirschberg, C. B. Effect of Nucleotides on Translocation of Sugar Nucleotides and Adenosine 3′-phosphate 5′-phosphosulfate into Golgi Apparatus Vesicles. *Biochim. Biophys. Acta* **1984**, *777*, 133−139.

(19) Chiaramonte, M.; Koviach, J. L.; Moore, C.; Iyer, V. V.; Wagner, C. R.; Halcomb, R. L.; Miller, W.; Melancon, P.; Kuchta, R. D. Inhibition of CMP-sialic Acid Transport into Golgi Vesicles by Nucleoside Monophosphates. *Biochemistry* **2001**, *40*, 14260−14267.

(20) Aoki, K.; Ishida, N.; Kawakita, M. Substrate Recognition by Nucleotide Sugar Transporters. *J. Biol. Chem.* **2003**, *278*, 22887−22893.

(21) Ishida, N.; Kawakita, M. Molecular Physiology and Pathology of the Nucleotide Sugar Transporter Family (SLC35). *Pfluegers Arch.* **2004**, *447*, 768−775.

(22) Milla, M. E.; Hirschberg, C. B. Reconstitution of Golgi Vesicle CMP-sialic Acid and Adenosine 3′-phosphate 5′-phosphosulfate Transport into Proteoliposomes. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1786−1790.

(23) Helmus, Y.; Denecke, J.; Yakubenia, S.; Robinson, P.; Luhn, K.; Watson, D.; McGrogan, P.; Vestweber, D.; Marquardt, T.; Wild, M. Leukocyte adhesion deficiency II patients with a dual defect of the GDP-fucose transporter. *Blood* **2006**, *107*, 3959−3966.

(24) Berninsone, P.; Eckhardt, M.; Gerardy-Schahn, R.; Hirschberg, C. B. Functional Expression of the Murine Golgi CMP-sialic Acid Transporter in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **1997**, *272*, 12616−12619.

(25) Guillen, E.; Abeijon, C.; Hirschberg, C. B. Mammalian Golgi Apparatus UDP-N-acetylglucosamine Transporter: Molecular Cloning by Phenotypic Correction of a Yeast Mutant. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 7888−7892.

(26) Abeijon, C.; Robbins, P. W.; Hirschberg, C. B. Molecular Cloning of the Golgi Apparatus Uridine Diphosphate-N-acetylglucosamine Transporter from *Kluyveromyces lactis*. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5963−5968.

(27) Ma, D.; Russell, D. G.; Beverley, S. M.; Turco, S. J. Golgi GDP-mannose Uptake Requires LPG2. A Member of a Eukaryotic Family of Putative Nucleotide-sugar Transporters. *J. Biol. Chem.* **1997**, *272*, 3799−3805.

(28) Selva, E. M.; Hong, K.; Baeg, G. H.; Beverley, S. M.; Turco, S. J.; Perrimon, N.; Hacker, U. Dual Role of the Fringe Connection Gene in Both Heparin Sulphate and Fringe-dependent Signaling Events. *Nat. Cell Biol.* **2001**, *3*, 809−815.

(29) Goto, S.; Taniguchi, M.; Muraoka, M.; Toyoda, H.; Sado, Y.; Kawakita, K.; Hayashi, S. UDP-sugar Transporter Implicated in Glycosylation and Processing of Notch. *Nat. Cell Biol.* **2001**, *3*, 816−822.

(30) Berninsone, P.; Hwang, H. O.; Zemtseva, I.; Horvitz, H. R.; Hirschberg, C. B. SQV-7, a Protein Involved in *Caenorhabditis elegans* Epithelial Invagination and Early Embryogenesis Transports UDP-glucuronic Acid, UDP-N-acetylgalactosamine and UDP-glucose. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3738−3743.

(31) Hoeflich, J.; Berninsone, P.; Goebel, C.; Gravato-Nobre, M. J.; Libby, B. J.; Darby, C.; Politz, D. M.; Hodgkin, J.; Hirschberg, C. B.; Baumeister, R. Loss of *srf*-3 Encoded Nucleotide Sugar Transporter Activity in *Caenorhabditis elegans* Alters Surface Antigenicity and Prevents Bacterial Adhesion. *J. Biol. Chem.* **2004**, *279*, 30440−30448.

(32) Caffaro, C. E.; Hirschberg, C. B.; Berninsone, P. Functional Redundancy of *Caenorhabditis elegans* Nucleotide-sugar Transporters. *FASEB J.* **2006**, *20*, A55.

(33) Hirschberg, C. B. Golgi Nucleotide Sugar Transport and Leukocyte Adhesion Deficiency II. *J. Clin. Invest.* **2001**, *108*, 3−6.

(34) Martinez-Duncker, I.; Dupre, T.; Piller, F.; Candelier, J. J.; Trinchet, C.; Tchemia, G.; Oriol, R.; Molicone, R. Genetic Complementation Reveals a Novel Human Congenital Disorder of Glycosylation of Type II, Due to Inactivation of the Golgi CMP-sialic Acid Transporter. *Blood* **2005**, *105*, 2671−2676.

(35) Thomsen, B.; Horn, P.; Panitz, F.; Bendixen, E.; Petersen, A. H.; Holm, L. E.; Nielsen, V. H.; Agerholm, J. S.; Arnbjerg, J.; Bendixen, C. A Missense Mutation in the Bovine SLC35A3 Gene, Encoding a UDP-N-acetylglucosamine Transporter, Causes Complex Vertebral Malformation. *Genome Res.* **2006**, *16*, 97−105.

(36) Herrero, A. B.; Uccelletti, D.; Hirschberg, C. B.; Dominguez, A.; Abeijon, C. The Golgi GDPase of the Fungal Pathogen *Candida albicans* Affects Morphogenesis, Glycosylation and Cell Wall Properties. *Eukaryotic Cell* **2002**, *1*, 420−431.
(37) Nishiwaki, K.; Kubota, Y.; Chigira, Y.; Roy, S. M.; Suzuki, M.; Schvarzstein, M.; Jigami, Y.; Hisamoto, N.; Matsumoto, K. An NDPase links ADAM Protease Glycosylation with Organ Morphogenesis in *C. elegans*. *Nat. Cell Biol.* **2004**, *6*, 31−37.
(38) D'Alessio, C.; Caramelo, J. J.; Parodi, A. Absence of Nucleoside Diphosphatase Activities in the Yeast Secretory Pathway Does Not Abolish Nucleotide Sugar-dependent Protein Glycosylation. *J. Biol. Chem.* **2005**, *280*, 40417−40427.
(39) Dean, N.; Zhang, Y. B.; Poster, J. B. The VRG-4 Gene is Required for GDP-mannose Transport into the Lumen of the Golgi in the Yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* **1997**, *272*, 31908−31914.
(40) Spath, G. F.; Lye, L. F.; Segawa, H.; Turco, S. J.; Beverley, S. M. Identification of a Compensatory Mutant (lpg2-REV) of Leishmania Major Able to Survive as Amastigotes Within Macrophages Without LPG2-dependent Glycoconjugates and its Significance to Virulence and Immunization Strategies. *Infect. Immun.* **2004**, *72*, 3622−3627.
(41) Herman, R. K.; Yochem, J. Genetic Enhancers (September 16, **2005**), *WormBook,* ed. The *C. elegans* Research community, WormBook, doi/10.1895/wormbook, 1.27.1, http://www.wormbook.org.
(42) Herman, T.; Horvitz, H. R. Three Proteins Involved in *Caenorhabditis elegans* Vulval Invagination are Similar to Components of a Glycosylation Pathway. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 974−979.
(43) Bulik, D. A.; Wei, G.; Toyoda, H.; Kinoshita-Toyoda, A.; Waldrip, W. R.; Esko, J. D.; Robbins, P. W.; Selleck, S. B. sqv-3, -7, -8, A Set of Genes Affecting Morphogenesis in *Caenorhabditis elegans*, Encode Enzymes Required for Glycosaminoglycan Biosynthesis. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 10838−10843.
(44) Cipollo, J. F.; Awad, A. M.; Costello, C. E.; Hirschberg, C. B. srf-3, A Mutant of *Caenorhabditis elegans*, Resistant to Bacterial Infection and to Biofilm Binding, is Deficient in Glycoconjugates. *J. Biol. Chem.* **2004**, *279*, 52893−52903.
(45) Hwang, H. Y.; Horvitz, H. R. The SQV-1 UDP-glucuronic Acid Decarboxylase and the SQV-7 Nucleotide Sugar Transporter May Act in the Golgi Apparatus to Affect *Caenorhabditis elegans* Vulval Morphogenesis and Embryonic Development. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 14218−14223.
(46) Abbreviations used: UDP-Gal, UDP-galactose; UDP-GlcNAc, UDP-*N*-acetylglucosamine; UDP-GalNAc, UDP-*N*-acetylgalactosamine; UDP-Glc, UDP-glucose; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; ATP, adenosine 5′-triphosphate.

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