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Nucleotide Sugar Transporters: Elucidation of Their Molecular Identity and Its Implication for Future Studies[1]

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Nucleotide sugar transporters are mainly located in the Golgi membranes and carry nucleotide sugars, that are produced outside the Golgi apparatus, into the organelle, where they serve as substrates for the elongation of carbohydrate chains by glycosyltransferases. They are thus indispensable for cellular glycoconjugate synthesis and, moreover, may have regulatory roles in producing the structural variety of cellular glycoconjugates. Their occurrence has long been well recognized, but studies on the molecular bases of their strict substrate specificities and modes of action have been hampered by the lack of information on their precise molecular structures. Complementary DNAs encoding several of these transporters were cloned recently, which represented a substantial step forward as to the above mentioned issues. The products of these cDNAs are mutually related hydrophobic proteins consisting of 320-400 amino acid residues with multiple putative transmembrane helix domains, and are located in the Golgi apparatus. This review briefly summarizes the present status of the field of nucleotide sugar transporter research, and also presents an outlook of the study in this field.

Key words: glycoconjugate, Golgi apparatus, modulation of glycosylation, molecular cloning, nucleotide sugar transporter.

The oligosaccharide units of glycoconjugates play a variety of biological roles (for an extensive list of references see Ref. *1*). These range from simple conformational and metabolic stabilization of proteins, to active participation in the processes of cell-matrix and cell-cell interactions by providing ligands for specific recognition. The bases for this versatility of the oligosaccharides are, on the one hand, the extensive variety in their structures and, on the other, the fact that a particular oligosaccharide structure is displayed in a well regulated manner at an appropriate time and a proper site in the body (*2*). Specific glycoconjugate structures thus appear and disappear in predetermined manners during the processes of development and differentiation, and in close association with tumor development and metastasis as well. To understand the molecular mechanisms underlying the production of this highly regulated variety of oligosaccharide structures, we have to first elucidate the molecular architecture of the machinery for glycoconjugate synthesis. Subsequently, the mechanism must eventually be described in terms of differential localization, mode of action and regulation of activity as well as of expression of the individual components that comprise the machinery.

Nucleotide sugar transporters and glycosyltransferases constitute two major groups of functional proteins, both of which are indispensable for glycoconjugate synthesis. The transporters are mainly located in the Golgi membranes and carry nucleotide sugars, that are produced outside the Golgi apparatus, into the organelle, where they serve as substrates for the elongation of carbohydrate chains by glycosyltransferases (*3*). These two groups of proteins are equally important for the overall reaction of glycoconjugate synthesis. Nevertheless, much less attention has been paid so far to nucleotide sugar transporters than to glycosyltransferases.

In recent years glycosyltransferases have been a subject of intensive studies (*4, 5*). This is partly because their differential and regulated expression has been expected to be mainly responsible for the generation of the extensive variety of cellular glycoconjugate structures. In contrast, it seems to be tacitly assumed that nucleotide sugar transporters are always active enough to provide glycosyltransferases with sufficient amounts of substrates, and that they

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Abbreviations: Fuc, fucose; Gal, galactose; GalNAc, *N*-acetylgalactosamine; Glc, glucose; GlcA, glucuronic acid; GlcNAc, *N*-acetylglucosamine; GS-II, *Glifonia simplicifolia* lectin II; Man, mannose; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; RACE, rapid amplification of cDNA ends; SA, sialic acid; UTR, untranslated region; WGA, wheat germ agglutinin; Xyl, xylose.

are consequently less important as elements contributing to the glycoconjugate diversity. These assumptions, however, may not be justified *a priori*. Different glycosyltransferases responsible for the formation of different isomeric linkages involving a given sugar residue are usually present in the Golgi membrane, and compete for the same nucleotide sugar as a common substrate. Supposing that the availability of a particular nucleotide sugar is limited in the Golgi lumen, the reactions of glycosyltransferases with higher affinity for that substrate would prevail, leading to the preferential synthesis of a limited variety of specific linkages. In this way, alterations in the activity of nucleotide sugar transporters may be of regulatory significance in producing a variety of patterns of cellular glycoconjugates, by changing the concentrations of nucleotide sugars inside the Golgi apparatus.

To determine whether or not nucleotide sugar transporters play any regulatory role in the generation of the well-controlled variety of specific glycoconjugate structures, it is important to have appropriate molecular probes for detecting and analyzing their expression, and the temporal as well as spatial variations in detail. In this context, we can say that the recent success in the cloning of cDNAs for several nucleotide sugar transporters has been a real breakthrough that has given us a starting point for inquiry into this challenging issue (*6-14*).

Needless to say, this molecular cloning is also an important step toward a better understanding of the molecular features of the nucleotide sugar transporters and basic mechanisms of the reaction they carry out, which is essential for obtaining a comprehensive picture of the process of cellular glycoconjugate synthesis. Based on these considerations, we describe in this article the molecular characteristics of nucleotide sugar transporters which have been revealed by cDNA cloning in some detail, and then discuss some prospects for future development of this field. Before doing so, however, a brief summary of earlier work in this field, which forms the basis for our current studies, is pertinent.

Earlier work on the nucleotide sugar transporters

For an overview of earlier work on the intracellular distribution and reactions of the nucleotide sugar transporters the readers are referred to a review by Hirschberg and Snyder published in 1987 (*3*). Further information may be found in shorter reviews which appeared more recently (*15, 16*).

Substrate-specific nucleotide sugar transporters and nucleotide sugar transporter-deficient mutant cells. Nucleotide sugars are synthesized in the cytosol with the sole exception of CMP-sialic acid (CMP-SA), which is synthesized in the nucleus, and then transported across the Golgi membranes (*17, 18*). They serve as the substrates of glycosyltransferases in the Golgi lumen, and their sugar moieties are transferred to a variety of specific oligosaccharides on glycoconjugates as acceptors. The transport reactions are mediated by highly specific transporters. This was suggested by earlier biochemical work indicating the absence of competition for a transporter between different nucleotide sugars, for instance, UDP-galactose (UDP-Gal) and UDP-glucose (UDP-Glc) (*19*), and then shown definitely on the isolation and characterization of nucleotide sugar transporter-deficient mutant cells. Thus, mutant cell lines, later characterized to be deficient in either CMP-SA transporter or UDP-Gal transporter, were found among those selected on the basis of altered cytotoxic lectin-sensitivities and other phenotypes, and were shown to have pleiotropic defects as to the addition of pertinent sugar residues to glycoproteins and glycolipids (*20-25*). Microsomal vesicles isolated from these cells were specifically unable to transport either CMP-SA or UDP-Gal, but were fully competent to transport other nucleotide sugars (*26, 27*). Some mutant cell lines derived from cancer cell lines were reported to exhibit decreased tumorigenicity or metastatic potential *in vivo* (*22, 25*). Interestingly, UDP-Gal transporter-deficient murine Had-1 cells were not only nontumorigenic in C3H/He mice, in contrast to parental FM3A carcinoma cells, but, furthermore, their prior inoculation into the mice suppressed the tumorigenicity of FM3A cells (*25*).

Intracellular localization of nucleotide sugar transporters. Nucleotide sugar transporting activities, including those for CMP-SA, UDP-Gal, and GDP-fucose (GDP-Fuc), are recovered almost exclusively in the Golgi membrane vesicle fraction in line with the current understanding that the glycosyltransfer reactions involving these substrates take place in the Golgi complex (*28, 29*). While several others, such as UDP-*N*-acetylglucosamine (UDP-GlcNAc), UDP-*N*-acetylgalactosamine (UDP-GalNAc), UDP-xylose (UDP-Xyl), and UDP-glucuronic acid (UDP-GlcA) transporting activities, are found in both the endoplasmic reticulum and Golgi-derived vesicles with a significantly larger proportion of the activities being recovered in the latter (*29-31*). Whether the transporters found in different organella represent distinct isoforms or a single molecular species may be somehow sorted to different membranes remains unknown. The physiological significance of the activities recovered in the endoplasmic reticulum membrane fraction is also obscure at present, since most of the glycosylation reactions involving these nucleotide sugars are supposed to take place in the Golgi apparatus. UDP-Glc transporting activity is mainly recovered in endoplasmic reticulum vesicles (*32*), which is quite reasonable in view of the important role played by UDP-Glc:glycoprotein glucosyltransferase (*33*) in the quality control of glycoproteins (*34-36*). GDP-mannose (GDP-Man) is not transported into mammalian Golgi membranes (*32*), but is transported into those of yeast and Leishmania (*10, 37*).

Mechanisms of the nucleotide sugar transport reaction. In the nucleotide sugar transport reaction in the Golgi membranes, as illustrated schematically in Fig. 1 for UDP-Gal, the whole substrate molecule is delivered to the lumen, which has been demonstrated clearly for GDP-Fuc by using substrates radiolabeled at the nucleotide and sugar moieties with different isotopes (*28*). The reaction is temperature-dependent with very little transport occurring at 0-4°C (*3, 28, 38*), and comprises electroneutral one-to-one exchange of a nucleotide sugar molecule for a corresponding nucleoside monophosphate molecule in its dianionic form (*39-42*). This was demonstrated biochemically, using rat liver Golgi membrane vesicles, by showing that the transport of UDP-GlcNAc in exchange for UMP was much faster at pH 7.50 than at pH 5.45, at which UMP^{2-} and UMP^{-}, respectively, occur as the major ionic species (*42*). Nucleoside monophosphate, which serves as the counter-substrate in this antiport process, is usually supplied continuously in the Golgi lumen through the

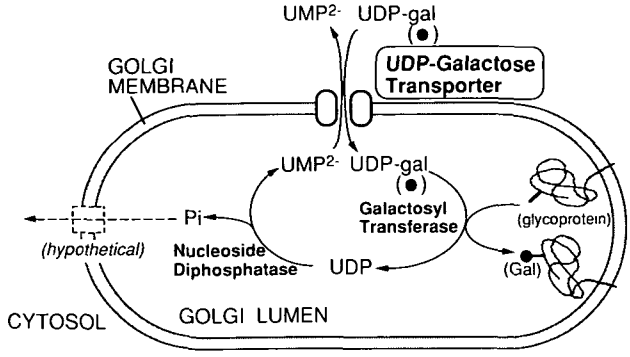

Fig. 1. **Mechanism of nucleotide sugar transporters.** See the text for details. UDP-sugars and GDP-sugars are transported in exchange for UMP and GMP, respectively, while CMP-SA is transported in exchange for CMP. Note that the pathway for the export of inorganic phosphate has not been identified yet and that the presence of a phosphate carrier remains hypothetical. −, nascent oligosaccharide chain; ●, galactose residue.

hydrolysis of nucleoside diphosphate, which is produced from nucleotide sugars as a result of ongoing glycosyltransfer reactions (*39-41*). Further support of this mechanism came from the fact that a yeast mutant with a defect in the Golgi guanosine diphosphatase gene (*gda1*) was found to be defective in the glycosylation of proteins and sphingolipids, and in the transport of GDP-Man into Golgi vesicles (*43, 44*). More recently, the overexpression of another yeast gene (*ynd1*), encoding a nucleoside diphosphatase with a broader substrate specificity, was found to complement the glycosylation defect of the *gda1* disruptant (*45*). Double disruptant (*Δgda1 Δynd1*) cells are not viable.

The K_m values for nucleotide sugars range between 1 to 10 μM (*28-32, 38*). Those for nucleoside monophosphates as counter-substrates in the antiport have not been measured directly, but the K_i values for competitive inhibition of the nucleotide sugar transport by nucleoside monophosphates have been measured and fall in the range of 1 to 5 μM (*46*). Nucleoside di- and triphosphates as well as 2′-deoxynucleotides are also inhibitory, while nucleoside 2′- and 3′-phosphates are not. Free sugars do not inhibit the transport (*46*). This is rather surprising in view of the strict specificity of the transporters for the sugar moieties. The reason for the lack of inhibition remains unclear.

Solubilization and reconstitution of the nucleotide sugar transport system. Nucleotide sugar transporters were solubilized from rat liver Golgi membranes, and then the transport systems were reconstituted in proteoliposomes (*47, 48*). The kinetic properties of the transporters were essentially unaltered in the reconstituted system as compared to those observed in the intact vesicles. Nucleoside 5′-monophosphates trapped in the liposomes during the reconstitution process stimulated the uptake of nucleotide sugars several fold in further support of the antiport mechanism mentioned above, but an absolute dependence on nucleoside 5′-monophosphate inside the liposomes has not been demonstrated.

The solubilization/reconstitution of the transport systems is one of the necessary steps for purification of nucleotide sugar transporters in functionally competent forms. Substantial purification was achieved for the transporter for phosphoadenosine phosphosulfate (PAPS) (*49*). PAPS is involved in the glycoconjugate synthesis as the sulfate donor for the formation of sulfated oligosaccharides, and its transport is mechanistically similar to that of nucleotide sugars in that it is transported in exchange for AMP. However, its reported molecular weight (70k) is considerably different from those of nucleotide sugar transporters (30k-40k) reported recently based on the nucleotide sequences of their cDNAs (*6-14*). Purification of other transporters has also been attempted through this approach (*48*), but extensive studies on the properties of nucleotide sugar transporters using purified preparations are still hampered by the limited quantities of the transporters naturally occurring in Golgi membranes.

Cloning of nucleotide sugar transporter cDNAs

As outlined above, we already know much about the characteristics of the nucleotide sugar transport systems and the reactions they carry out, and are also beginning to unveil their molecular identities through partial purification and reconstitution. Nevertheless, we have still much to learn about the structural bases for their reactivity as well as the regulation of their expression and activity in order to fully elucidate their physiological role in exquisitely controlled glycoconjugate synthesis. In this context, a spate of reports dealing with the cDNA cloning of several nucleotide sugar transporters has certainly had the effect of accelerating the studies in this field (*6-14, 38, 50, 51*).

The nucleotide sugar transporter family. The list of cDNAs cloned so far includes those for human and fission yeast UDP-Gal transporters (*6, 7, 11*), human, murine, and Chinese hamster CMP-SA transporters (*7, 9, 12, 14*), budding yeast UDP-GlcNAc transporter (*8*), and Leishmania and budding yeast GDP-Man transporters (*10, 13*). The cDNA clones for these nucleotide sugar transporters were identified on the basis of their competence to complement the phenotypic defects of mutant cells devoid of ability to transport the respective nucleotide sugars. In the case of human UDP-Gal transporter cDNA, for example, mouse Had-1 cells served as the recipients of the cDNA (*6, 7*). Had-1 was originally isolated as a Newcastle disease virus receptor-deficient mutant strain derived from mouse FM3A cells, and was later determined to be deficient in UDP-Gal transporter (*23, 24*). The mutant shows an altered lectin-sensitivity spectrum as compared to that of the parental cells. Thus, Had-1 is $WGA^rGS\text{-}II^s$ (WGA, wheat germ agglutinin; GS-II, *Glifonia simplicifolia* lectin II), while FM3A is $WGA^sGS\text{-}II^r$ (*24*). Introduction of the human UDP-Gal transporter cDNA into Had-1 cells rendered the cells $WGA^sGS\text{-}II^r$. The biological activity of human CMP-SA transporter was recently confirmed in a similar way by monitoring the change in WGA-sensitivity of CHO-derived Lec2 cells on introduction of the candidate cDNA (*14*). The murine CMP-SA transporter and yeast UDP-GlcNAc transporter cDNAs were identified on the basis of the appearance of specific sugar residues on cell surface glycoconjugates, that could be detected as the binding of appropriate lectins, using a fluorescence-activated cell sorter (FACS), on their introduction into mutant cells deficient in pertinent transporters, namely Lec2 cells and the *mnn-2* mutant of *Kluyveromyces lactis*, respectively (*8, 9*). A *Schizosaccharomyces pombe* mutant, *gms1-1*, which lacks UDP-Gal transporter, and a *Leishmania donovani* mutant, C3PO (*lpg2*$^-$), and a *Saccharo-*

myces cerevisiae mutant, *vrg4-2*, which are defective in GDP-Man transporter, were utilized to identify the genes for the respective transporters (*10, 11, 13*).

The cloning of these cDNAs supported the idea that there should be a number of mutually related nucleotide sugar transporters which differ in their substrate specificities. These cDNAs encode a group of novel mutually related proteins (see below) of which structural homologues were not found in the databases of well defined genes, with just a few putative proteins inferred from the genomic DNA sequences of yeast and nematode showing significant similarity (*6, 8, 9*).

A further search of the DNA sequence databases revealed information concerning several additional related genes in the "Expressed Sequence Tags" database (dbEST) (*7, 10*). In many cases, only incomplete and tentative sequence information acquired from truncated cDNA clones may be available in this database, but the information has merit in that it originates from mRNAs which were definitely expressed in the cells. The information obtained from the database turned out to be useful in that it facilitated characterization of the nucleotide sugar transporter gene family. In fact, several truncated cDNA clones were extended to cover their full-lengths, and their nucleotide sequences completed to yield the structures of human CMP-SA transporter and three closely related putative nucleotide sugar transporters of man, mouse, and rat, most likely sharing an as yet unidentified substrate (*7*).

The structural relationship among nucleotide sugar transporters and their relatives, including some putative ones, is summarized in Fig. 2. The dendrogram suggests that they can be divided into three subgroups based on the degree of sequence similarity, UDP-Gal and CMP-SA transporters, GDP-Man transporter, and UDP-GlcNAc transporter being representatives of the three subgroups, but the possible functional as well as evolutionary significance of this remains to be elucidated. It is interesting that UDP-Gal transporters are more like CMP-SA transporters than UDP-GlcNAc transporters.

Isoforms of UDP-Gal transporter. The human UDP-Gal-transporter actually occurs in two isoforms, hUGT1 and hUGT2 (*6, 7*). They were detected among the population of TIG-1 human normal diploid fibroblast cell mRNAs during an attempt to extend a truncated cDNA clone, selected from a TIG cell cDNA library, toward the 3′-end according to the 3′-RACE procedure. Subsequent analysis indicated that they share the entire 5′-UTR as well as most of the ORF portion in common, but that the C-terminal 5 amino acid residues and the entire 3′-UTR of hUGT1 were replaced in hUGT2 by a different stretch of 8 amino acid residues and a distinct 3′-UTR sequence which was much shorter than that of hUGT1 (*7*). Sequence analysis of the human UGT gene, which is mapped to band Xp11.22-p11.23 on the X chromosome (*52*), revealed that hUGT1 and hUGT2 mRNAs may be produced from the same gene through alternative splicing (*7*).

Both hUGT1 and hUGT2 cDNAs were able to correct the $WGA^{r}GS\text{-}II^{s}$ phenotype of Had-1 cells, rendering them $WGA^{s}GS\text{-}II^{r}$ (*7*). Microsomal vesicles prepared from Had-1- and *S. cerevisiae*-transformants expressing either hUGT1 or hUGT2 cDNA were active in UDP-Gal transport with practically the same K_m for UDP-Gal (*7, 38*). It is intriguing as to whether or not the expression of these

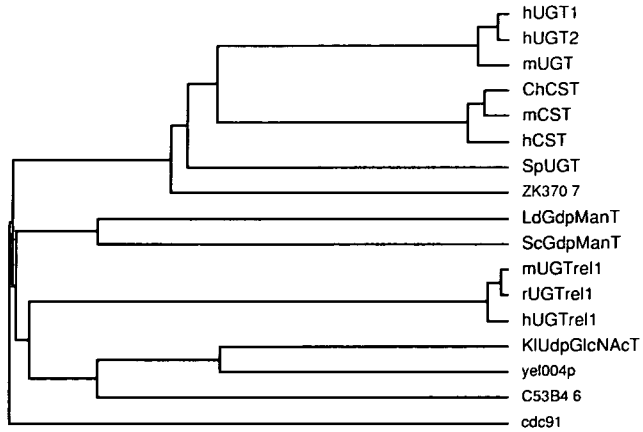

Fig. 2. **Dendrogram illustrating relationships among nucleotide sugar transporters and putative products of some nucleotide sugar transporter-related genes.** The clustering of related sequences occurred with a series of pairwise alignments with the UPGMA (unweighted pair-group method using arithmetic averages) algorithm, using the PileUp program (Wisconsin Sequence Analysis Package, Genetic Computing Group, Madison, WI, USA). The distance along the vertical axis is proportional to the difference between sequences. The similarity between hUGT1 and cdc91 calculated with the BESTFIT program (Genetic Computing Group) is 43%. hUGT1 and hUGT2, human UDP-Gal transporters 1 and 2, respectively (*6, 7*); m and SpUGT, murine and *S. pombe* UDP-Gal transporters, respectively [(*11*), Ishida, N. *et al.*, unpublished results]; Ch, m and hCST, Chinese hamster, murine and human CMP-SA transporters, respectively (*9, 12, 14*); Ld and ScGdpManT, *L. donovani* and *S. cerevisiae* GDP-Man transporters, respectively (*10, 13*); m, r, and hUGTrel1, murine, rat, and human UGT-related gene 1 products, respectively (*7*); KlUdpGlcNAcT, *K. lactis* UDP-GlcNAc transporter (*8*); ZK370.7 and C53B4.6, nucleotide sugar transporter-related putative proteins of *C. elegans* (*7*); yel004p and cdc91, nucleotide sugar transporter-related putative proteins of *S. cerevisiae* (*6, 7*).

isoforms is differentially regulated spatially and temporally among various tissues and during development. The choice of appropriate poly A addition sites sometimes constitutes the basis for the regulation of gene expression (*53, 54*). It should be noted in this context that 3′-UTR of the hUGT1 mRNA contains three mRNA-destabilizing AUUUA sequences (*6*). Isoforms have so far not been found for other nucleotide sugar transporters.

Structural features of nucleotide sugar transporter cDNAs

Nucleotide sugar transporter cDNAs code for proteins consisting of 320–400 amino acid residues, which exhibit 40–60% similarity in amino acid sequence with each other. The proteins are highly hydrophobic and are predicted to be intrinsic membrane proteins with multiple (5 to 9) putative transmembrane helix domains (*6–12*). Illustrated in Fig. 3, as an example, is the possible structure of hUGT1, which is predicted to have 8 putative transmembrane helices.

Unfortunately, besides these overall structural features, alignment of the sequences of these related proteins found so far does not seem to allow further generalization to relate their particular structural features to specific functions. Some have a leucine zipper motif in their sequences (*6, 8, 9, 12*), but others do not (*10, 11*). Leucine zippers have been proposed to serve as oligomerization signals in other

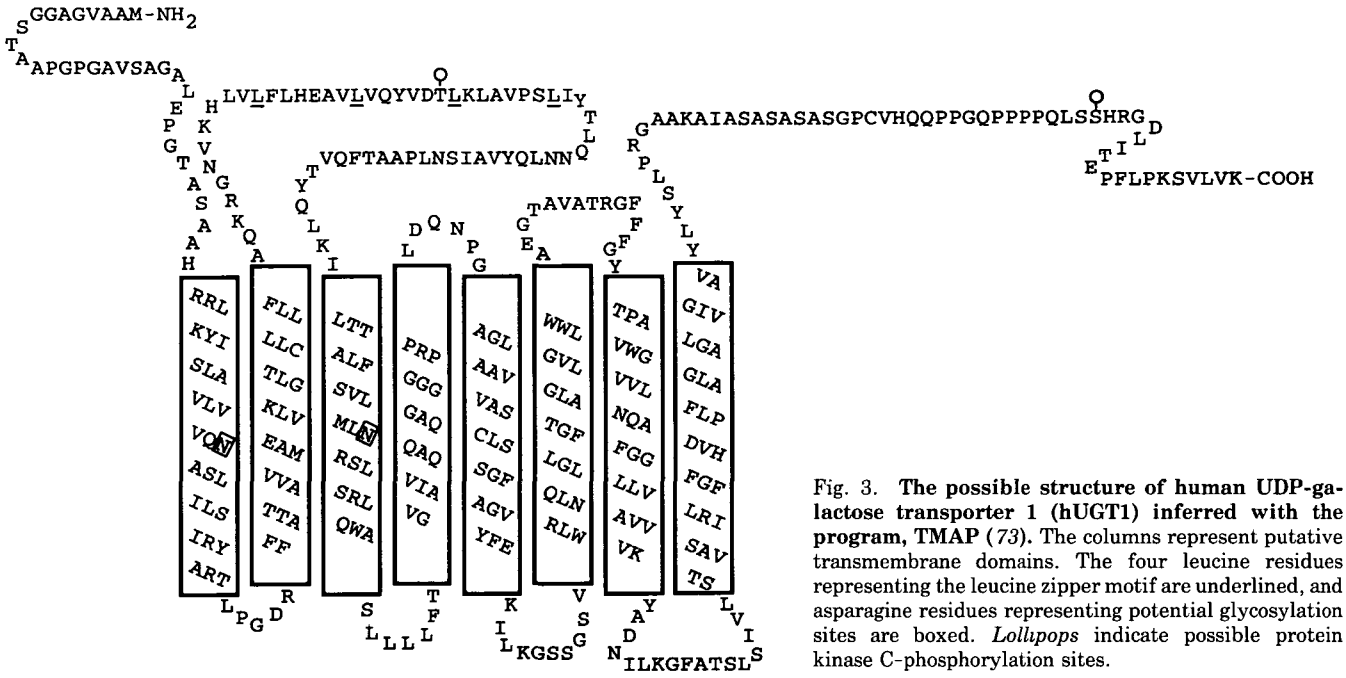

Fig. 3. **The possible structure of human UDP-galactose transporter 1 (hUGT1) inferred with the program, TMAP** (*73*). The columns represent putative transmembrane domains. The four leucine residues representing the leucine zipper motif are underlined, and asparagine residues representing potential glycosylation sites are boxed. *Lollipops* indicate possible protein kinase C-phosphorylation sites.

proteins (*55, 56*), and this motif is also found in acetyl-CoA transporter (*57*), but their significance in the nucleotide sugar transporters remains controversial. Potential sites for asparagine-linked glycosylation are present in the sequences, but there has been no indication that the proteins are in fact glycoproteins. Some of them are actually located in presumed transmembrane helices (*6*). The fact that hUGT1 and hUGT2 expressed in *S. cerevisiae* showed the same mobility on SDS-PAGE as that expressed in Had-1 as well as the endogenous one detected in HeLa cells may imply that the proteins are not glycosylated (*38, 50*). Possible protein kinase C-phosphorylation motifs are also found in the putative cytoplasmic domain (*6*). Partial similarity to glycosyltransferases sharing a given nucleotide sugar as the common substrate might be expected, but actually was not found. Structural features suggestive of a possible nucleotide binding domain have not been observed either. Additional information on the structures of other family members may help us identify common structural features relevant to their transporter function, but complementary information obtained through biochemical and reverse genetical studies is definitely needed to elucidate the structure-function relationship of nucleotide sugar transporters.

Expression of nucleotide sugar transporter cDNAs

These cDNAs in fact code for the transporter proteins *per se*, not regulatory or supplementary factors that may indirectly affect their expression, stability, or activity. This was demonstrated directly by showing the heterologous expression of human UDP-Gal transporter and murine CMP-SA transporter in *S. cerevisiae*, which is innately not equipped with these transporters (*38, 51*). Expression on the zero-background can be observed in these systems, and microsomal membranes were shown to actively transport these nucleotide sugars. The transport reactions were substrate-specific and saturable, with apparent K_m values comparable with those obtained with mammalian Golgi membranes. The reactions were dependent on temperature and the integrity of membrane vesicles, and were inhibited by specific nucleoside monophosphates (*38, 51*).

The hUGT2 product expressed in yeast was recovered in the Golgi membrane fraction, as well as in a heterogeneous organellar membrane fraction derived from vacuoles and the endoplasmic reticulum (*38*). The hUGT protein expressed in non-Golgi membranes also seemed to be transport-competent (*38*). These expression systems will be valuable in future studies for obtaining purified transporter proteins, which will in turn be reconstituted in liposomes. This will provide a conclusive answer as to whether or not they are in fact able to carry out the transport reaction by themselves.

Nucleotide sugar transporter cDNAs were also introduced into homologous mammalian, protozoan, and yeast cells, and the activities and localization of the transporters were investigated (*7–14, 50*). In mammalian and protozoan cells, transporters were clearly shown to be sorted correctly to the Golgi membranes, as described below. Microsomal membranes containing Golgi-derived membrane vesicles were prepared from nucleotide sugar transporter-deficient cells, transformants expressing nucleotide sugar transporter cDNAs, and transport-competent parental cells, and examined for their ability to transport nucleotide sugars. In every case examined, (human and fission yeast UDP-Gal transporters (*7, 11*), human CMP-SA transporter (*14*), budding yeast UDP-GlcNAc transporter (*8*), and Leishmania and budding yeast GDP-Man transporter (*10, 13*)), the vesicles obtained from revertant cells recovered the ability to transport a pertinent nucleotide sugar, that was lacking in those from corresponding mutant cells, to a level comparable with or a little higher than that in the parental cells. In human UDP-Gal transporter cDNA-expressing Had-1 cells and human CMP-SA transporter cDNA-expressing Lec2 cells, immunoblotting analysis involving

peptide antibodies demonstrated the occurrence of a specific cDNA product in microsome vesicles (*14, 50*). Membrane vesicles were further fractionated on a Percoll density gradient, and the cDNA product as well as the UDP-Gal transporting activity was shown to be colocalized with a Golgi membrane marker, β1,4-galactosyltransferase, while being separated from NADPH-cytochrome *c* reductase, a marker for the endoplasmic reticulum (*50*).

Immunofluorescence microscopy with antibodies raised against C-terminal peptides of human UDP-Gal and CMP-SA transporters revealed the transporter proteins in the Golgi complex region of Had-1-derived and Lec2-derived transformants expressing the products of the respective cDNAs (*14, 50*). It is important that the antibodies also stained the Golgi region of HeLa cells, clearly indicating that the protein is an intrinsic Golgi membrane component (*50*). Epitope-tagged products of the human and murine CMP-SA transporter (*9, 14*), and Leishmania and yeast GDP-Man transporter cDNAs (*10, 13*) were also detected in the Golgi complex region on either immunofluorescence microscopy or immunoelectron microscopy.

Further information concerning the orientation of the transporter molecules relative to the Golgi membranes is emerging through further immunocytochemical experiments, and the results indicate that the C-terminal portion of hUGT1 as well as that of human CMP-SA transporter is faced to the cytosol on the external surface of the Golgi membrane (*14*). This orientation is in accordance with that predicted previously (*6, 12*) (see also Fig. 3). On the other hand, the epitope-tag at the C-terminus of Leishmania GDP-Man transporter was localized by the immunogold technique to the lumenal side of the Golgi membrane (*10*). Further studies are definitely needed to determine in more detail the orientation of the transporters relative to the membrane.

What shall we learn next?

Structure-function relationship of the nucleotide sugar transporters and their basic reaction mechanisms. Inspection of the published amino acid sequences of nucleotide sugar transporters does not tell us much about their functionally important domains, as explained above. Determination of the structures of mutant genes will help us to obtain clues as to which part of the transporter molecule is important. Preliminary results indicate that UGT mRNA from Had-1 cells codes for a peptide truncated at the middle of the coding region of the parental mRNA (Ishida, N., unpublished result). This is the first direct indication that the UGT gene provides the basis of the nucleotide sugar (UDP-Gal) transport deficient phenotype of Had-1 cells. Analysis of mutants belonging to the same complementation group (*23*) will yield further information concerning the structural requirements for the UDP-Gal transporting activity.

The determinant of the substrate specificity is another critical issue that is totally obscure at present. Information to be obtained through domain swapping experiments on UDP-Gal and CMP-SA transporters may turn out to be instructive. These transporters can be expressed in the Golgi membranes of cultured cells, and easily detected with specific antibodies (*9, 14, 50*). Consequently, one may readily examine the activity of chimeric molecules between these transporters using appropriate transporter-deficient mutant cells.

Purification of nucleotide sugar transporters will become much easier than before with the success in cDNA cloning. Progress has been made in the construction of efficient expression systems, including heterologous ones, which may be used in place of the rat liver as the source of Golgi membranes enriched with a particular transporter (*14, 38, 50, 51*). Specific antibodies against these transporters are already available and may turn out to be valuable for purification of the recombinant transporters. Rigorous studies on the substrate specificity will be possible only after proteoliposomes reconstituted from such purified recombinant transporters become available. It remains to be seen whether or not the residual glycosylation in the deficiency of a given nucleotide sugar transporter represents a compensatory function of any other transporter primarily designed for delivering another substrate. Moreover, with a purified transport system we may study the possible interaction between the transporters and auxiliary factors that may modulate the transport reactions. Elucidation of the precise reaction mechanisms also awaits purification of the transporters in sufficient quantities.

The signals and mechanisms for sorting of nucleotide sugar transporters to the correct locations. The enzymes involved in glycoconjugate synthesis, including glycosyltransferases and glycosidases, are located in different subcompartments of the Golgi complex, and are arranged, in general, in the order in which they act to form an appropriate oligosaccharide structure (*58*). The same general rule also seems to apply to nucleotide sugar transporters, as earlier biochemical work suggested (*3, 16*), but the mechanisms for their targeting to the proper sites are not known. Intensive studies on the signals and mechanisms directing glycosyltransferases to their proper locations in the Golgi cisternae have been reviewed recently (*59*). The situation is rather complicated, because general Golgi retention signals represented by common linear amino acid sequences were not found in these type II membrane proteins with a large lumenal catalytic domain, although the importance of the transmembrane domains was evident (*59*). Two possible models for Golgi retention mechanisms, namely, the bilayer thickness model (*60, 61*) and the oligomerization/kin recognition model (*62, 63*), have been proposed for these type II membrane proteins, but to what extent they are applicable to polytopic membrane proteins such as nucleotide sugar transporters remains to be seen.

The Golgi-targeting mechanism for polytopic Golgi membrane proteins has not yet been defined at all, but may be conserved at least partly between yeast and higher eukaryotes, since a significant portion of the hUGT2 protein expressed in *S. cerevisiae* was targeted correctly to the Golgi membranes (*38*). It is interesting that mammalian glycosyltransferases as such have not so far been successfully targeted to the yeast Golgi membranes in heterologous expression systems (*64*), although chimeric proteins consisting of the membrane anchor region of yeast Golgi type II membrane protein fused to the catalytic domain of mammalian glycosyltransferase, and the membrane anchor region of mammalian glycosyltransferase fused to an appropriate soluble reporter protein could be targeted to the yeast Golgi membranes (*65, 66*). The analysis of possible localization signals and the mechanisms of target-

ing will constitute an important part of future studies on nucleotide sugar transporters.

Studies on glycosylation enzymes indicated that their localizations overlap and that their subcompartmentation in the Golgi region shows cell-type specific differences (*59*). Although definitive evidence is lacking as to whether or not this is also true for nucleotide sugar transporters, it is possible that such flexibility in subcompartmentation constitutes a common feature of the components of the glycosylation system. If this is the case, it may have a profound physiological implication. Rigorous analysis of the subcompartmentation of nucleotide sugar transporters using appropriate specific probes is therefore needed.

Availability of substrates as determinants of cellular glycoconjugate structure. UDP-Gal transporter-deficient cells show a pleiotropic aberrance in the structures of both cell surface glycoproteins and glycolipids (*21, 24, 25*). A drastic reduction in galactosylation as a result of a severe shortage of the substrate leads to a decrease in the amounts of sialylated glycoconjugates and lactosylceramide with a concomitant increase in the amounts of GlcNAc-terminated sugar chains and glucosylceramide (*24, 25*). It should be noted, however, that the galactosylation at different linkages is not uniformly affected by defective UDP-Gal transport. Intensive analysis of asparagine-linked oligosaccharides of FM3A and Had-1 cells revealed that Galβ1-4GlcNAc linkages amounting to 10-15% of those in the parental cells persisted in the mutant cells, whereas Galα1-3Gal linkages were totally absent in the mutant (*24*). The incorporation of galactose residues into the link tetrasaccharides of proteoglycans, which is also believed to occur in the Golgi complex (*31*), seems to be surprisingly resistant to the deficiency of UDP-Gal, since the amounts of chondroitin sulfate and heparan sulfate were not reduced significantly in UDP-Gal transporter-deficient cells derived from CHO and MDCK cells (*67, 68*). Keratan sulfate, that contains Gal residues in its glycosaminoglycan moiety, is sensitive to a UDP-Gal deficiency (*68*). The simplest but not the sole explanation for the differential sensitivity of isomeric galactoside linkages to a substrate deficiency is that the K_m values for UDP-Gal of individual galactosyltransferases involved in the synthesis of these linkages are different from each other, and the one with a higher K_m value is more sensitive to lowering of the substrate concentration in the Golgi lumen.

Modulation of glycoconjugate structure through control of nucleotide sugar transport. The above consideration raises the possibility that the structures of cellular glycoconjugates can be modulated through the activity of transporters delivering the substrates to the sites of oligosaccharide chain elongation and modification. The temporal and spatial variation in the expression of the nucleotide sugar transporters and their isoforms is a matter of particular interest in this respect. The modulation can be positive as well as negative. In fact, a putative acetyl-CoA transporter cDNA was successfully cloned by monitoring the increase in the amount of *O*-acetylated ganglioside expressed on the surface of specially designed recipient cells (*57*). It may also be relevant that Had-1/hUGT1 and Had-1/hUGT2 transformants showed significantly higher WGA-sensitivity than the parental FM3A cells (*6, 7*).

It should be pointed out that the possibility of controlling Leishmaniasis by specifically inhibiting the GDP-Man transport in *L. donovani* is conceivable (*10*). Inhibition of GDP-Man transport would suppress lipophosphoglycan synthesis, which is implicated in its virulence (*69*), while GDP-Man transporter is not essential for humans. The fact that the Galα1-3Gal linkage seems to be highly sensitive in mouse cells to a reduction in the substrate concentration may also be noteworthy (*24*). This galactoside linkage is a constituent of the so called α-galactosyl epitope, which is present in most mammals, but absent from humans and Old World primates, and recently has been a matter of considerable discussion in relation to xenotransplantation and retroviral-mediated gene therapy (*70*).

An ongoing project intending the remodelling of yeast-type oligosaccharides into mammalian-type ones would be an extreme case of modulation of glycoconjugate structure in which changes in the nucleotide sugar transporter level are involved. The production in yeast of recombinant glycoproteins with human-type oligosaccharides is necessary to obviate problems, including the strong antigenicity, reduced functional potency, and pharmacokinetic instability of these products, which are inherent to the administration in practice of heterologously produced glycoproteins. A mutant strain of *S. cerevisiae* (*Δoch1 mnn1*) which accumulates *N*-linked glycans with a structure corresponding to that of the core oligosaccharide synthesized in the endoplasmic reticulum of mammalian cells was engineered (*71*), and recombinant yeast strains expressing mammalian glycosyltransferases were constructed (*64, 66*). The functional expression of mammalian UDP-Gal and CMP-SA transporters in yeast is very significant in this respect (*38, 51*). In fact, the galactosylation of cell-surface *N*- and *O*-linked oligosaccharides has been demonstrated recently in the *Δmnn1* mutant of *S. cerevisiae*, in which both hUGT2 and *S. pombe* α1,2-galactosyltransferase cDNAs are expressed (*72*). This may represent a significant step toward the production of human-type oligosaccharides in yeast. Future studies may disclose additional possibilities and examples of the nucleotide sugar transporter-mediated control of glycoconjugate expression.

The cloud of frustration stemming from the lack of specific probes has lifted with the success in molecular cloning of nucleotide sugar transporters, and we are now equipped with powerful tools that will help us to deal with a number of challenging issues in this and related fields. Solutions to the problems and tasks, both basic and applicative, outlined above will constitute the material for another review on this subject, which we hope will be written in the not too distant future.

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