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The role of nucleotide sugar transporters in development of eukaryotes

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Abstract

The Golgi apparatus membrane of all eukaryotes has nucleotide sugar transporters which play essential roles in the glycosylation of glycoproteins, proteoglycans and glycolipids. Mutations of these transporters have broad developmental phenotypes across many species including diseases in humans and cattle.

Keywords

Golgi apparatus; Nucleotide sugar transporter; Glycosylation; Development; Diseases

1. Introduction

Defects in glycosylation cause very diverse developmental phenotypes that are shown in Fig.1. Panel A is of a child with growth and mental retardation and abnormal toes [1]. Panel B corresponds to a calf with a short neck, fused vertebrae and scoliosis [2]. Panel C shows a mutant *Drosophila melanogaster* that has a shorter leg and a nicked wing [3]. Panel D shows a mutant *Caenorhabditis elegans* with abnormal gonad migration [4].

These four diverse phenotypes have in common defects in glycosylated macromolecules. Specifically, there is a deficiency of covalent linkages between sugars and proteins and/or between sugars and other sugars which in turn are bound to proteins. Defects also occur in glycolipids where sugars are covalently linked to lipids. The mechanisms leading to these biochemical defects originate from mutations in specific **nucleotide sugar transporters**, membrane proteins present in the Golgi apparatus of all eukaryotes. Recent reviews, emphasizing biochemical aspects of these transporters have been published [5–7].

1.1. Why is there a need for Golgi apparatus nucleotide sugar transporters?

Approximately half of all proteins in eukaryotic cells are either membrane bound or secreted. Both groups of proteins are synthesized on membrane bound polysomes, then translocated into the lumen of the endoplasmic reticulum from where they are transported, via vesicles, to the Golgi apparatus. Thereafter, these proteins are either transported, via vesicles, to other organelles, such as the plasma membrane or lysosomes, or are secreted to

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the outside of cells. In the lumen of the endoplasmic reticulum and Golgi apparatus approximately eighty percent of secreted and membrane bound proteins undergo posttranslational modifications, principally glycosylation but also sulfation and phosphorylation.

Substrates for these modifications are activated sugars (nucleotide sugars), activated sulfate (adenosine 3'-phosphate 5'-phosphosulfate) and activated phosphate (ATP). None of these nucleotide derivatives are synthesized in the lumen of the Golgi apparatus, where the above modifications occur, but rather in the cytosol. Exceptions to this rule are ATP, most of which is synthesized in the mitochondria, and CMP-sialic acid, which is synthesized in the nucleus, for an unknown reason. Because the site of synthesis of these nucleotide derivatives is different from the luminal Golgi apparatus compartment where they act as substrates, the need for a mechanism of translocation of these substrates into the luminal Golgi apparatus compartment arises. This led to the search and discovery of Golgi apparatus membrane nucleotide sugar transporters [5–7].

1.2. Nucleotide sugar transporters are antiporters

Nucleotide sugar transporters may transport, in a saturable manner, one or several substrates, depending on the specific transporter. When more than one substrate is transported by a specific transporter, transport may be competitive, e.g. relatively high concentrations of one substrate inhibit transport of the other substrate(s) [8] or simultaneous and independent [4,6]. Examples of nucleotide sugar transporters using one substrate have been reported in mammals, yeast, *Entamoeba* and *Giardia* while multiple substrate transporters have been reported so far in mammals, *D. melanogaster*, *C. elegans*, *Leishmania* and plants. The affinity (K_m) of nucleotide sugars for their transporters is generally in the 1–10 micromolar range. The transporters are antiporters with the corresponding nucleoside monophosphates, e.g. UMP for uridine-diphosphate nucleotides and GMP for guanosine-diphosphate nucleotides [5–7].

As will be discussed in a later section, mutants in nucleotide sugar transporters have biochemical phenotypes, in addition to some of the morphological ones shown in Fig. 1. Early studies with cells in tissue culture and yeast and later with multicellular organisms, showed that glycoproteins, proteoglycans and glycolipids were deficient in those sugars whose corresponding nucleotide sugar transport activity was impaired [6].

1.3. Transport of nucleotide sugars regulates glycosylation of macromolecules

Evidence supports the hypothesis that transport of nucleotide sugars into the Golgi apparatus regulates which macromolecules become glycosylated in the lumen. A mutant MDCK cell line which is 95% deficient in transport of UDP-Gal showed, as expected reduced galactosylation of glycoproteins, glycosphingolipids and proteoglycans. Interestingly, among different proteoglycans, only biosynthesis of keratan sulfate, which contains galactose in its polymer, was affected while levels of chondroitin- and heparan- sulfate, proteoglycans that only contain galactose in their linkage region but not in their polymer, were not reduced. One could hypothesize that the K_m for the linkage galactosylation is lower than that for polymer synthesis. This would favor galactosylation of the linkage region when supply of UDP-Gal is limiting in the Golgi apparatus lumen, as occurs in the MDCK mutant. Another possibility may be subcompartmentation of transporters in the Golgi apparatus membrane. This may result in different galactose-containing macromolecules being synthesized in these compartments due to selective availability of UDP-Gal in these subcompartments. Evidence consistent with this hypothesis has been obtained in *Leishmania major* where subcompartmentation has been shown to occur with glycosyltransferases.

Thus, select availability of nucleotide sugars for different glycosyltransferases may also provide a mechanism for regulation of glycosylation of macromolecules.

Another site of possible regulation of nucleotide sugar transport, and thus macromolecular glycosylation, is in the generation of nucleoside monophosphates, the antiporters for nucleotide sugar transport. It has been previously shown in *S. cerevisiae* mutants, lacking the Golgi apparatus GDPase, that a limited supply of luminal GMP allows for selective synthesis of some mannose-containing glycoconjugates. Finally as will be discussed in a later section, functional redundancy between nucleotide sugar transporters in different tissues, may also contribute to selective macromolecular glycosylation.

1.4. Nucleotide sugar transporters that share significant amino acid sequence identity may have different substrate specificities while those with little identity may have the same substrate specificity

The amino acid sequence of nucleotide sugar transporters has been determined from many species. One of the most important general characteristics, in addition to being very hydrophobic proteins that form homodimers and cross the membrane of the Golgi apparatus six to ten times, is the fact that one cannot easily determine the substrate specificity of nucleotide sugar transporters based on *in silico* predictions. For example, transporters with approximately 50% amino acid sequence identity may have different substrate specificities (Fig. 2). On the other hand, transporters with amino acid sequence identity as low as 20% may have the same substrate specificity (Fig. 2). Because of this, many assigned substrate specificities in databases are not correct. Only experimentally determined substrate specificity results are reliable. For this an important tool is expression of genes encoding putative transporters in *S. cerevisiae* or *Giardia*, as these two species have the advantage of solely having two or one endogenous nucleotide sugar transport activities, respectively. Following isolation of Golgi apparatus enriched vesicles from these organisms, transport saturability of different nucleotide sugars is measured into vesicles from transformed and non transformed cells. This will allow one to determine the occurrence of non-endogenous transport activities. The latter activities are most likely the result of the heterologous transporters being expressed. When possible, substrate specificity of putative transporters should also be corroborated by phenotypic correction of mutants previously shown to have a defect in a specific nucleotide sugar transporter.

Several nucleotide sugar transporters have been purified to apparent homogeneity. This required the reconstitution of these transporters into artificial vesicles, called liposomes, followed by measurements of transport activities into them. To date no crystal structure of nucleotide sugar transporters of the Golgi membrane has been reported.

The next sections will deal with the role of these transporters in higher and lower eukaryotes. In humans and *C. elegans* for example, there are seven sugars in the glycoconjugates of these organisms. Yet, based on *in silico* predictions, these organisms appear to have 17–18 putative nucleotide sugar transporters. The fact that several of these transporters can transport multiple nucleotide sugars and that some of the transporters show partial overlap in their tissue expression [6], illustrates a major level of complexity that needs to be resolved before the roles of these transporters in multicellular organisms can be understood in depth.

2. Mammalian diseases caused by mutations in nucleotide sugar transporters

2.1. Leukocyte Adhesion Deficiency II: A mutation in the human GDP-fucose transporter

Fucosylation is a common modification of *N*- and *O*-linked glycans and has been shown to participate in many biological events such as developmental and immune defense mechanisms [9]. Leukocyte Adhesion Deficiency Syndrome II (LADII), also called Congenital Disorder of Glycosylation IIc, is an autosomal recessive disorder. This disease is characterized by severe growth and mental retardation, the Bombay blood group phenotype (which is the blood group H antigen lacking fucose), and a primary immunodeficiency with recurrent infections due to defects in neutrophil chemotaxis (Fig.1A) [10]. Golgi enriched vesicles from fibroblasts or lymphoblasts from LADII patients showed a significantly lower (although not a complete absence of) transport activity of GDP-Fuc into Golgi apparatus derived vesicles while transport of other nucleotide sugars was normal [11,12]. In more recent studies it was shown that the lower transport activity was in fact the result of mutations in the GDP-Fuc transporter, that render it partially active. Therefore other transporters in the Golgi membrane must not necessarily assume GDP-Fuc transport in order to account for the biochemical phenotype.

Two independent groups cloned the human GDP-Fuc transporter and a summary of partial loss of function mutations found to date in this transporter is shown in Table 1 [13,14]. An in depth study of the glycoconjugates resulting from the R147C mutation showed that fucosylation was greatly reduced in *N*- but not *O*-glycans, suggesting a role of nucleotide sugar transporters in regulation of glycosylation reactions [15]. Interestingly, dietary supplementation of fucose selectively increased fucosylation of some patients but not others [1,12,16]. At present it is not clear whether or not this different responsiveness is the result of distinct mutations of the GDP-Fuc transporter affecting either the affinity (Km) for the nucleotide sugar or the Vmax for transport into the Golgi apparatus or both.

A mouse knockout model of the GDP-Fuc transporter showed many phenotypic characteristics previously found in LADII [17]. However there was a small amount of residual fucose linked to glycoconjugates in tissues as well partial restoration of this deficiency upon dietary fucose supplementation. Whether or not this is the result of an additional GDP-Fuc transporter in mice or whether another nucleotide sugar transporter may transport GDP-Fuc, albeit, less efficiently than the wild type, is not clear.

2.2. Schneckenbecken Dysplasia: A mutation in the UDP-Glucuronic acid /UDP-N-Acetylgalactosamine transporter

A mouse knockout of nucleotide sugar transporter SLC35D1, which transports UDP-GlcA acid and UDP-GalNAc, resulted in a neonatal lethal form of skeletal dysplasia affecting cartilage and skeletal development [18]. Severe shortening of limbs and facial structures was found as well as impairment in the biosynthesis of chondroitin sulfate but not of heparan sulfate. The similarities between the knockout mouse phenotype and human Schneckenbecken dysplasia, an autosomal recessive trait, resulted in identification of a loss of function mutation in the corresponding human gene. A more recent study identified different mutations in this transporter in five different families with the above dysplasia but not in other spondylodysplastic dysplasias (Table 1) [19].

2.3. Complex Vertebral Malformation: A cattle disease with a mutation in the UDP-N-Acetylglucosamine transporter

Complex Vertebral Malformation (CMV) is a recessive genetic disorder that was first described in 2001 and has been found in cattle in the United Kingdom, Netherlands, United

States, Japan and Denmark [20]. In these latter two countries approximately 30 percent of the Holstein cattle population are heterozygotic carriers. The disease causes aborted or malformed calves with phenotypes that vary from subtle to severe, including axial skeletal deformities such as fused and misshapen vertebrae, symmetric arthrogryposis of the lower limb joints and craniofacial dysmorphism (Fig. 1B). Genetic analyses showed that the CVM calves have an identical allele locus containing 20 known genes, including the UDP-GlcNAc transporter among them [2]. The authors postulated that a mutation in this transporter may be responsible for the CVM phenotype. This was confirmed by finding a G to T transversion in the UDP-GlcNAc transporter gene resulting in a valine to phenylalanine substitution at position 180 (Table 1). Genetic complementation with a mutant strain of the yeast *K. lactis* confirmed that this mutation resulted in a loss of transporter function. Nevertheless, human complex vertebral malformation has so far not revealed mutations in the above homologous transporter gene [21].

3. Nucleotide sugar transporters during development of non mammals

3.1. The role of nucleotide sugar transporters in the development of *Caenorhabditis elegans* and novel lessons learned

The study of nucleotide sugar transporters in *C. elegans* has led to important new concepts regarding the role of glycosylation in multicellular organisms as well as novel biochemical characteristics of these transporters. Epithelial invagination in multicellular organisms is a crucial process for the formation of tubular structures during gastrulation and organogenesis such as vulva formation. An elegant genetic screen for *C. elegans* mutants with a squashed vulva (sqv) phenotype revealed that eight genes, SQV 1 through 8, are involved in vulval epithelial invagination [22]. Because all eight gene products are involved in different aspects of glycosylation, this study represents a classic example for the significance of glycosylation in regulating tissue development. Specifically, SQV-3 and SQV-8 are glycosyltransferases [23] and SQV-4 is a UDP-Glc dehydrogenase responsible for producing UDP-GlcA [24].

SQV-7 encodes a nucleotide sugar transporter, the first of these transporters shown to regulate tissue development in this nematode and the first to be shown to transport multisubstrates [8]. To date 18 putative nucleotide sugar transporter encoding genes are predicted in *C. elegans* of which four have been characterized for their substrate specificity as shown in Table 2. Studies of nucleotide sugar transporters in this nematode were also the first to reveal their tissue specific expression as summarized in Table 2. Transport of multiple nucleotide sugars by SQV-7 is competitive, e.g. high concentrations of one substrate inhibit transport of another. Mutants in this gene showed decreased levels of proteoglycans, consistent with the multisubstrate specificity of this transporter [8,25].

SRF-3 was identified in a screen for ectopic binding with the wheat germ agglutinin lectin, which does not occur in wild type nematodes [26]. The changes in surface antigenicity of the mutant conferred resistance to pathogenic bacteria. Consistent with the substrate specificity of the transporter for UDP-Gal, biochemical analyses showed significant reduction of O- and N-linked galactose-containing glycoconjugates in the mutants [27,28].

Transporter ZK986.9 transports UDP-Glc, UDP-Gal, UDP-GlcNAc and UDP-GalNAc [58]. No obvious morphological phenotype is detected upon knockdown of gene expression by RNAi [58].

In more recent studies, while RNAi of transporter C03H5.2 in wild type nematodes only caused disorganization of body fibers, silencing of this gene in *srf-3* mutants resulted in an accumulation of oocytes in proximal gonad arms and aberrant gonad migration (Fig.1D) [4]. Because there is partial tissue and substrate specificity overlap between transporters

C03H5.2 and SRF-3, the above results suggest functional redundancy among nucleotide sugar transporters in multicellular organisms. Biochemical analyses of glycoconjugates of SRF-3 mutants in which C03H5.2 expression had been drastically reduced by RNAi were not performed. However, it is likely that the biosynthesis of glycoconjugates was altered in the mutants suggesting that these macromolecules play an important role in the above described developmental events. As previously mentioned, this is particularly relevant to other organisms such as humans which also have more putative nucleotide sugar transporters than sugars in their glycoconjugates. Biochemical studies of SRF-3 and C03H5.2 showed a novel transport mechanism for multiple nucleotide sugars, namely simultaneous and independent. With both SRF-3 and C03H5.2, excess of one substrate does not inhibit the translocation of the other, contrary to previously found with SQV-7 [4,29]. Future structural studies of the SRF-3 and C03H5.2 transporters should reveal the mechanism of this novel transport.

3.2. The role of nucleotide sugar transporters in *Drosophila melanogaster* development

Two nucleotide sugar transporters have been identified in *D. melanogaster*, *fringe connection* (*frc*) and *Gfr*. *Frc* was initially identified in two separate genetic screens for mutations affecting cuticle segment polarity and limb development [3,30]. Inhibition of expression of this gene at early developmental stages caused embryonic death and segment polarity phenotypes while inhibition at post embryonic stages caused developmental defects including aberrant wing morphology, fused short legs and reduced rough eyes (Fig.1C) [3,30]. Loss of *Gfr* function resulted in segmentation and wing phenotypes, including nicked wings and extra wing margins, indicative of cell signaling defects [31,32]. *Gfr* has been shown to encode a GDP-Fuc transporter [31,33]. However the substrate specificity of *frc* is equivocal. One study found UDP-GlcA, UDP-GlcNAc and UDP-xylose as substrates [3]; the other study reported the above first two substrates in addition to UDP-GalNAc, UDP-Gal and UDP-Glc [30]. While the latter study showed no evidence for substrate saturation, the former showed only one substrate saturation. Neither study performed phenotypic correction of a UDP-GlcNAc mutant. Taken together, these studies illustrate the importance of rigorous analyses in determining substrate specificity for nucleotide sugar transporters.

The mutant phenotypes described above are typical of mutants in the Notch signaling pathway. This led three groups [3,30,31] to investigate the role of the nucleotide sugar transporters in this signaling pathway. Several lines of evidence support such a role: (a) Chemical analyses showed that *Frc* and *Gfr* are required for the biosynthesis of the Notch receptor. (b) Mutations in *Frc*, *Gfr* and *Fringe* (*Fng*)-dependent Notch pathway have similar phenotypes, most likely as a result of Notch disruption. (c) The occurrence of genetic interactions between *Frc*/*Gfr* and Notch/*Fng* mutants. Details of Notch glycosylation are covered in the accompanying article in this issue.

Moreover, biochemical analyses showed that heparan sulfate proteoglycans were greatly reduced in *frc* mutants [3]. This proteoglycan plays a key role in activating signaling pathways such as Wingless (Wg/Wnt), Hedgehog (Hh) and FGF receptor during *Drosophila* development [34,35]. Biosynthesis of the above proteoglycan was also impaired in a mutant of the Golgi apparatus 3'-phosphoadenosine 5'-phosphosulfate transporter gene (*slalom*), which supplies this key substrate for proteoglycan biosynthesis to the Golgi apparatus lumen [36]. In summary, the above transporters for nucleotide sugars and nucleotide sulfate play an essential role by providing necessary substrates into the Golgi apparatus lumen for the biosynthesis of heparan sulfate proteoglycans and biosynthesis of Notch receptor during *Drosophila* development.

4. The role of nucleotide sugar transporters in the growth and pathogenicity of lower eukaryotes

Nucleotide sugar transporters have been identified in yeast, fungi, trypanosomatids, *Giardia* and *Entamoeba*. Vesicles from *Entamoeba* were isolated and shown to transport nucleotide sugars. This demonstrated the conservation of higher eukaryotic protein secretory pathways, including Golgi apparatus functions such as glycosylation of proteins in lower organisms [37]. As will be discussed below, mutations of genes encoding nucleotide sugar transporters in lower eukaryotes have important phenotypes. Differences in substrate specificities between transporters in higher and lower eukaryotes may open the possibility for therapeutic approaches.

4.1. The role of nucleotide sugar transporters in the pathogenicity of *Leishmania*

Leishmania species synthesize and secrete glycoconjugates such as lipophosphoglycan (LPG) and proteophosphoglycan (PPG), which are polymers containing disaccharides of galactose and mannose-phosphate [38]. These glycoconjugates have been shown to have important roles in parasite survival, virulence and infectivity to their mammalian hosts. The GDP-Man transporter, encoded by the LPG2 gene, was identified in three *Leishmania* species: *L. major*, *L. mexicana* and *L. donovani*. This transporter from the latter organism also transports GDP-Fuc and GDP-arabinose [39]. Deletion of LPG2 in all three species resulted in a global deficiency of phosphoglycan-containing molecules [40–43]. All three null strains were susceptible to lysis by complement *in vitro* [44,45]. Most interestingly, infectivity to their mammalian hosts differed among the mutant strains. *LPG2*-null *L. major* and *L. donovani* lost their ability to cause acute pathology in the infectious mouse model, while *LPG2*-null *L. mexicana* retained virulence and induced infection of mice [40,42,44].

LPG5A and LPG5B are genes encoding UDP-Gal transporters in *L. major*. Mutants with a knockout of either gene alone still synthesized phosphoglycans. Deletion of LPG5A affected the synthesis of lipophosphoglycan but not proteophosphoglycan while deletion of LPG5B had the opposite effect. Deletion of both genes resulted in complete loss of phosphoglycans, suggesting that these genes have overlapping but not identical functions [46]. In *L. major* a double mutant of LPG5A and LPG5B still retained infectivity and virulence in mice despite a lack of phosphoglycans [47]. This suggests that other glycoproteins, unrelated to phosphoglycans, may be involved in virulence.

Protozoan parasites and fungi require translocation of GDP-Man into their Golgi apparatus lumen for synthesis of their mannoproteins. This differs from their mammalian hosts, which do not have a GDP-Man transporter. The fact that the mammalian hosts do not have GDP-Man transport activity, makes this transporter an attractive target for potential anti-fungal and anti-parasitic therapeutics. In addition, avirulent *L. major* *lpg2*⁻ has been shown to survive at a constant low level without causing disease but inducing protective immunity in mice. This so called “persistence without pathology” may be of value in generation of live, attenuated vaccines [42,48,49].

4.2. The role of nucleotide sugar transporters in yeast and fungal growth

Several yeast species have outer mannan chains which are assembled in the lumen of the endoplasmic reticulum and Golgi apparatus. A gene encoding a Golgi apparatus GDP-Man transporter which is essential for viability has been described in *S. cerevisiae* (*ScVrg4*), *Candida albicans* (*CaVrg4*) and *Candida glabrata* (*CgVrg4*) [50–52]. Partial loss of function mutations of *ScVrg4* resulted in decreased growth rates, aberrant shapes and sporulation. Mutations of *CgVrg4* also resulted in abnormal phenotypes including

pseudohyphae, reduced mannans and increased sensitivity to antibiotics such as hygromycin [52].

The human fungal pathogen *Cryptococcus neoformans* and the filamentous fungus *Aspergillus nidulans*, both possess two GDP-Man transporters Gmt1/Gmt2 and GmtA/GmtB, respectively [53,54]. Deletion of either Gmt1 or Gmt2 in *C. neoformans* did not cause lethality. Deletion of *Gmt1* caused capsule integrity defects, which was not observed upon deletion of Gmt2, suggesting that both transporters have different biological roles [55]. A missense mutation of GmtA in *A. nidulans*, resulted in hyphal morphological defects and changes in cell wall properties [53]. Together the above results indicate an important role for the GDP-Man transporters in both *C. neoformans* and *A. nidulans*.

A mutant in the UDP-GlcNAc transporter of the yeast *K. lactis* showed only biochemical but not morphological or growth phenotypes. The mutant proved most valuable in enabling the cloning of the first nucleotide sugar transporter of any species [56].

5. Future directions

Studies of nucleotide sugar transporters in the future can be envisioned to cover two broad areas: biological and structural. One important biological direction will be to continue with studies on substrate specificity and tissue distribution of different nucleotide sugar transporters in multicellular organisms such as *C. elegans* and *D. melanogaster*. These studies should further clarify the role of different transporters in the development and physiology of these organisms. Most likely additional examples of functional redundancy will be found which should have conceptual relevance to mammalian physiology. Humans have a similar number of putative nucleotide sugar transporters as those of *C. elegans*, and both have only seven sugars in their glycoconjugates. One should also explore the possibility that non glycoconjugates are being affected as a consequence of secondary and tertiary effects on expression of other genes resulting from mutations in nucleotide sugar transporters. Transcriptional and translational regulation of expression of the genes encoding nucleotide sugar transporters is another area that needs to be studied as well as how these parameters affect glycosylation in physiological and pathological states. In terms of human health, the development of specific inhibitors of the GDP-Man transporter, which is present in many pathogenic lower eukaryotes but not in mammals, could represent a unique strategy to treat and combat diseases caused by these organisms.

Structural and biochemical studies on transporter proteins are important future directions. The crystal structures of transporters will need to be obtained, particularly to determine the mechanism by which transport of multiple nucleotide sugars may occur in an independent and simultaneous manner. The use of chimeras as well as cross linking with appropriate nucleotide sugars should shed light on the mechanism by which transporter proteins recognize their substrates. This is important as primary amino acid sequence has not allowed accurate predictions of substrate specificity of nucleotide sugar transporters, as discussed in previous sections. This has resulted in inaccurate annotation of substrate specificities of putative nucleotide sugar transporters in the databases.

As presented in this review studies on the biology of these transporters have led to new concepts on the role of glycoconjugates during development in eukaryotes as well as new strategies to combat disease. Therefore future studies on the biology and biochemistry of these transporters should further shed light on novel biological and biochemical paradigms in these organisms.

Abbreviations

UDP-Gal	Uridine diphosphate galactose
UDP-Glc	Uridine diphosphate glucose
UDP-GlcA	Uridine diphosphate glucuronic acid
UDP-GlcNAc	Uridine diphosphate- <i>N</i> -acetylglucosamine
UDP-GalNAc	Uridine diphosphate- <i>N</i> -acetylgalactose
GDP-Fuc	guanosine diphosphate fucose
GDP-Man	guanosine diphosphate mannose
MDCK	Madin-Darby canine kidney
LADII	Leukocyte adhesion deficiency type II
SQV	Squashed Vulva.

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Fig. 1.

The phenotypes of nucleotide sugar transporter diseases and mutations in multicellular organisms. **A.** A child with Leukocyte Adhesion Deficiency II (a mutation of the GDP-Fuc transporter), Copyright Elsevier (J Pediatrics) (1999) reference [1]. **B.** A calf with complex vertebral malformation (a mutation in the UDP-GlcNAc transporter), Copyright Cold Spring Harbor Laboratory Press, reference [2]. The malformed vertebrae and scoliosis are shown by arrows, the fused ribs are shown by arrowheads. **C.** *Drosophila melanogaster* with a mutation in a multisubstrate nucleotide sugar transporter, adapted by permission from Macmillan Publishers Ltd. reference [3]. Arrowheads show the shortened joints between tarsal segments in the *frc* mutant. Nicked wing is indicated by an arrow. **D.** *Caenorhabditis*

C. elegans nucleotide sugar transporter mutant *srf-3* in which the nucleotide sugar transporter C03H5.2 was silenced, Copyright ASBMB (2007), reference [4]. The migration of nogads is depicted by white arrows.

UDP-GlcNAc-T_Kl -----MSFVLILSLVFGCCSNVISFE
 UDP-GlcNAc-T_Canis -MSTN-----LKYLSLGILVFQTTSLVLTMRYSRT
 UDP-GlcNAc-T_Bovine -MSAN-----LKYLSLGILVFQTTSLVLTMRYSRT
 UDP-Gal-T_Human -MAAVGAGGSTAAPGPGAVSAGALEPGTASAAHRRKLYISLAVLVQNASLILSIRYART
 CMP-SA-T_Human -MAAP-----RDNVTLLFKLYCLAVMTLMAAVYTIALRYTRT
 GDP-Fuc-T_Human MNRAPLKRSRILHMAITGASDPSAEAEANGEKPFLLRALQIALVVSPLYWVTSISMVFLNK

 UDP-GlcNAc-T_Kl HMVQGSNINLGNIVTFTQFVS---VTLIQLPNALDFSHPFRLRPRHIPLKIHMLAVFL
 UDP-GlcNAc-T_Canis LKEEGPRYLSSTAVVVAELLKIMACILLVYKDSKCSLRALNRILHDEILNKPMTLKLAI
 UDP-GlcNAc-T_Bovine LKEEGPRYLSSTAVVVAELLKIMACILLVYKDSKCSLRALNRILHDEILNKPMTLKLAI
 UDP-Gal-T_Human LP--GDRFFATTAVVMAEVLKGLTCLLLFAQKRGNVKHLVFLHEAVLVQYVDTLKLAV
 CMP-SA-T_Human SD--KELYFSTTAVCITEVIKLLLSVGILAKET-GSLGRFKASLRENVLGSPKELLKLSV
 GDP-Fuc-T_Human YLLDSPSLRLDTPFIFVTFYQCLVTTLCKGLSALAACCPGAVDFPSLRDLRVARSVLPL

 UDP-GlcNAc-T_Kl FFTSSVANNVSFKFDIS---VPIHIIIRCSGTTLTMIIGWAVCNKRYSKLQVQSAITMTL
 UDP-GlcNAc-T_Canis PSGIYTLQNNLLYVALSNLDAATYQVTTYQLKILTALFSVSMLSKKLGQVLSLVILMT
 UDP-GlcNAc-T_Bovine PSGIYTLQNNLLYVALSNLDAATYQVTTYQLKILTALFSVSMLSKKLGQVLSLVILMT
 UDP-Gal-T_Human PSLIYTLQNNLQYVAISNLPAAFTQVTTYQLKILTALFSVLMNRSLSRLQWASLLILFT
 CMP-SA-T_Human PSLVYAVQNNMAFLALSNLDAAVYQVTTYQLKIPCTALCTVLMNRTLKQLQWVSVMFLCA
 GDP-Fuc-T_Human -SVVFIGMITFNNLCLKYVGVAFINVGRSLTTVFNVLLSYLLLKQTTSFYALLTCGIIG

 UDP-GlcNAc-T_Kl GAIVASLYRDKEFSMDSLKLNTDSVGMTQKSMFGIFVVLVATALMSLLSLLNEWTYNKG
 UDP-GlcNAc-T_Canis GVAFFVQWPSDSQE-----LDSKELSAGSQFVGLMAVLTACFSSGFAGVYFEKILKETK
 UDP-GlcNAc-T_Bovine GVAFFVQWPSDSQE-----LNSKELSAGSQFVGLMAVLTACFSSGFAGVYFEKILKETK
 UDP-Gal-T_Human GVAIVQAQQAGG-----GGPRPLDQ-NPGAGLAAVVASCLSSGFAGVYFEKILKGSS
 CMP-SA-T_Human GVTLVQWKPAQA-----TKVVVEQNPLLGFGAIAIAVLCSGFAGVYFEKVLKSSD
 GDP-Fuc-T_Human GFWLGVDDQ-----EGAEGTLSWLGTVFGVLASLCVSLNAIYTTKVLPAVD

 UDP-GlcNAc-T_Kl KHWKETLFYSHFLALPLFMLGYTRLRDEFDRDLLISSDSMDIPIVKLPIATKLFMLIANV
 UDP-GlcNAc-T_Canis QSVWIRNIQLGFFGSIFGLMGVYIYDDELVSCKNG-----FFQGYNRLTWIVVLQAL
 UDP-GlcNAc-T_Bovine QSVWIRNIQLGFFGSIFGLMGVYIYDDELVSCKNG-----FFQGYNRLTWIVVLQAL
 UDP-Gal-T_Human GSVWLRNLQLGLFGTALGLVGLWVAEGTAVATRG-----FFFGYTPAVWGVVLNQAF
 CMP-SA-T_Human TSLWVRNIQMYLSGIIIVTLAGVYLSDGAEIKEKG-----FFGYTYTYVWFVIFLASV
 GDP-Fuc-T_Human GSIWRLTFYNNVNACILFLP-LLLLLGLGELQALRD-----FAQLGSAHFWMGMTLGGI

 UDP-GlcNAc-T_Kl TQFICIKGVNMLASNTDALTLSSVLLVRKFVSLLS-VYIYKNVLSVTAYLGTITVFLGA
 UDP-GlcNAc-T_Canis G---GLVIAAVIKYADNILKGFATSLSIILSTLISYFWLQDFVPTSVFFLGAILVITAT
 UDP-GlcNAc-T_Bovine G---GLVIAAVIKYADNILKGFATSLSIILSTLISYFWLQDFVPTSVFFLGAILVITAT
 UDP-Gal-T_Human G---GLLVAVVVKYADNILKGFATSLSIIVLSTVAS-IRLFGFHVDPFLFALGAGLVIGAV
 CMP-SA-T_Human G---GLYTSVVVKYTDNIMKGFSAAAAIVLSTIAS-VMLFGLQITLTFALGTLLVCVSI
 GDP-Fuc-T_Human FGFAIGYVTGLQIKFTSPLTHNVSGTAKACAQTVLA-VLYYEETKSFLWWTNNMVLGGS

 UDP-GlcNAc-T_Kl GLYSYG-----SVKTALPR-----
 UDP-GlcNAc-T_Canis FLYGYD-----PKPTGNP-----TKA--
 UDP-GlcNAc-T_Bovine FLYGYD-----PKPAGNP-----TKA--
 UDP-Gal-T_Human YLYSLPRGAAKAIASASASASGPCVHQPPGQPPPPQLSSHRGDLITEPFLPKLLTKVKG
 CMP-SA-T_Human YLYGLPRQD-----TTSIQOQGETASK-----ERVIG
 GDP-Fuc-T_Human SAYTWVRG-----WEMKKTPEEPSPKDSE-----KSAMG

Fig. 2.
 Amino acid sequence alignment of UDP-GlcNAc transporter from *Kluyveromyces lactis* (AAC49313), UDP-GlcNAc transporter from *canis* (NP_001003385), UDP-GlcNAc transporter from *Bos taurus* (NP_001098856.1), UDP-Gal transporter from *Homo sapiens* (NP_005651.1), CMP-sialic acid transporter from *Homo sapiens* (NP_006407.1) and GDP-Fuc transporter from *Homo sapiens* (NP_060859.4). The Clustal W method was used. Identical amino acids are in black.

Table 1

Mammalian nucleotide sugar transporter diseases

Diseases	Genes	Gene mutations	Protein mutations	Ethnic origins	References
Leukocyte Adhesion deficiency (CD11c)	GDP-fucose transporter	Transversion C439 to G	Missense T308R	Arabic	[59]
		Transition C923 to T	Missense T308R	Turkish	[13,14]
		Deletion G588	Nonsense, Truncation/open reading frame shift (after S195)	Brazilian	[60]
		Transition G969 to A	Nonsense, Truncation (after L322)	Pakistani	[61]
Complex Vertebral malformation	UDP-N acetyl-glucosamine Transporter	Transversion G538 to T	Missense V180F	Cattle	[2]
Schneckenbecken dysplasia	UDP-N acetyl-galactosamine transporter	Deletion A125	Nonsense, Truncation/open reading frame shift (after S42)	N.A.	[18]
		Transition G932 to A	Nonsense, Truncation (after W311)	N.A.	[18]
		Transversion/insertion, IVS7+1G to T	Nonsense, Truncation/open reading frame shift (after K212)	N.A.	[18]
		Transition C319 to T	Nonsense, Truncation (after R107)	Caucasian/American	[19]
		Transversion/insertion, IVS4+3A to G	Nonsense, Truncation/open reading frame shift (after L109)	Caucasian/American	[19]
		Deletion/exon skipping, deletion of 103bp from 534 to 636	Nonsense, Truncation/open reading frame shift (after R178)	Turkish	[19]
		Transversion A139 to C	Missense, T65P	Turkish	[19]

Diseases	Genes	Gene mutations	Protein mutations	Ethnic origins	References
CDG IIc	CMP- sialic acid transporter	Transition IVS6+1G to A	Nonsense, Truncation/open reading frame shift (After S108)	N.A.	[62]
		Deletion 130bp of exon 6	Nonsense, Truncation/open reading frame shift (After S327)	N.A.	[62]

N.A., not available

Table 2

Nucleotide sugar transporters of *Caenorhabditis elegans*: Biological phenotypes and biochemical substrate specificities

Nucleotide sugar transporters	SQV-7 [8,23,25,57]	SRF-3 [27,28]	C03H5.2 [4,29]	ZK986.9 [58]
Substrate specificity	UDP-Gal UDP-GalNAc UDP-GlcA	UDP-Gal UDP-GlcNAc	UDP-GalNAc UDP-GlcNAc	UDP-Gal UDP-GalNAc UDP-GlcNAc UDP-Glc
Tissue expression pattern	Vulval, Seam cells, Oocytes	Pharyngeal gland cells, Seam cells,	Pharyngeal gland cells, Seam cells, Spermatheca Vulval, Body wall muscle, Stomatointestinal muscle	N.D. *
Morphological Phenotype of mutant and /or RNAi	Squashed vulva Abnormally small Oocytes Gonad defect Hermaphrodite sterility	Wheat germ agglutinin surface binding	RNAi in wild-type worms: no obvious morphological changes. C03H5.2 RNAi in <i>stf-3</i> strain: oocyte accumulation abnormal gonad migration	No abnormal Phenotypes detected
Biochemical phenotypes of loss of function mutants	Reduced chondroitin and heparin sulphate	Reduced O- and N-linked glycoconjugates	N.D.	N.D.
Transport properties	Competitive non-cooperative	Simultaneous non-competitive	Simultaneous non-competitive	N.D.

* N.D.: Not determined