Molecular characterization of human UDP-glucuronic acid/UDP-*N*-acetylgalactosamine transporter, a novel nucleotide sugar transporter with dual substrate specificity

Masatoshi Muraoka, Masao Kawakita*, Nobuhiro Ishida

Department of Physiological Chemistry, The Tokyo Metropolitan Institute of Medical Science (Rinshoken), 18-22 Honkomagome 3-chome, Bunkyo-ku, Tokyo 113-8613, Japan

Received 1 December 2000; revised 6 March 2001; accepted 15 March 2001

First published online 30 March 2001

Edited by Felix Wieland

Abstract A novel human nucleotide sugar transporter (NST) which transports both UDP-glucuronic acid (UDP-GlcA) and UDP-*N*-acetylgalactosamine (UDP-GalNAc) has been identified, cloned and characterized. The strategy for the identification of the novel NST involved a search of the expressed sequence tags database for genes related to the human UDP-galactose transporter-related isozyme 1, followed by heterologous expression of a candidate gene (hUGTrel7) in *Saccharomyces cerevisiae* and biochemical analyses. Significantly more UDP-GlcA and UDP-GalNAc were translocated from the reaction medium into the lumen of microsomes prepared from the hUGTrel7-expressing yeast cells than into the control microsomes from cells not expressing hUGTrel7. The possibility that this transporter participates in glucuronidation and/or chondroitin sulfate biosynthesis is discussed.

Key words: Nucleotide sugar transporter; UDP-glucuronic acid; UDP-*N*-acetylgalactosamine; Endoplasmic reticulum; Glucuronidation; Chondroitin sulfate biosynthesis

1. Introduction

Cellular glycoconjugates, including glycoproteins, glycolipids and proteoglycans, are glycosylated in various ways in the endoplasmic reticulum (ER) and the Golgi compartment. The catalytic center of most glycosyltransferases involved in these glycosylation processes faces the lumen of these organelles. Therefore, a variety of nucleotide sugars, the monosaccharide donor in oligo/polysaccharide chain elongation, have to be transported from the cytosol into the lumen of the ER and the Golgi apparatus by specific transporters [1,2].

Analysis of such transporters that contribute to glycoconjugate biosynthesis in mammalian cells has led to the cloning and characterization at the molecular level of UDP-galactose (UDP-Gal) [3], CMP-sialic acid (CMP-Sia) [4–6] and UDP-*N*-acetylglucosamine (UDP-GlcNAc) transporter cDNAs [7,8], including UDP-Gal transporter isozyme cDNAs [9]. These transporters are localized in the Golgi membrane, and provide substrates utilized in the synthesis of Asn-linked oligosaccharides and glycolipids. In UDP-Gal transporter-deficient mouse Had-1 cells, glycosylation of glycoproteins and glycolipids is severely impaired [10].

In this report, we describe the isolation of a cDNA whose existence came to our notice through a search of the expressed sequence tags database (dbEST) for genes related to the human UDP-Gal transporter-related isozyme 1 [9]. Heterologous expression of the open reading frame (ORF) of this cDNA in the yeast *Saccharomyces cerevisiae* revealed that it encodes a novel transporter that translocates both UDP-glucuronic acid (UDP-GlcA) and UDP-*N*-acetylgalactosamine (UDP-GalNAc) from the cytosol into microsomal membrane vesicles. Transporters that act on UDP-GlcA and UDP-GalNAc, which are responsible for delivering substrates utilized in biologically important reactions such as glucuronidation and the synthesis of the glycosaminoglycan moiety of chondroitin sulfate, have not previously been cloned. Identification of the nucleotide sugar transporters (NSTs) involved in these biosynthetic processes would greatly help in elucidating the mechanisms and regulation of these processes. The gene product was expressed in the ER in mammalian cells. Based on the substrate specificity and the subcellular localization, the possibility that this transporter participates in glucuronidation and/or chondroitin sulfate biosynthesis is discussed.

2. Materials and methods

2.1. Materials

The radioactive nucleotide sugars and 3′-phosphoadenosine 5′-phosphosulfate (PAPS) were purchased from American Radiolabeled Chemicals, except that UDP-[4,5-^{3}H]Gal was purchased from NEN Life Science Products.

2.2. Yeast strain, transformation and culture

S. cerevisiae strain YPH500 (*MATα ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1*) was used in the expression study. Yeast transformation was performed using the lithium acetate method as described previously [11]. Transformants were selected on a selective medium containing 0.67% (w/v) Bacto-yeast nitrogen base without amino acids, 2% glucose (YNBD), and auxotrophic supplements except uracil. For preparation of membrane vesicles, the transformants were grown in liquid selective medium until they reached a density of about 0.8A_{600}. Cupric sulfate was then added to the culture at a final

*Corresponding author. Fax: (81)-3-3823 2965.
E-mail: kawakita@rinshoken.or.jp

Abbreviations: ER, endoplasmic reticulum; UDP-Gal, UDP-galactose; UDP-GlcNAc, UDP-*N*-acetylglucosamine; UDP-GalNAc, UDP-*N*-acetylgalactosamine; UDP-GlcA, UDP-glucuronic acid; CMP-Sia, CMP-sialic acid; GDP-Man, GDP-mannose; PAPS, 3′-phosphoadenosine 5′-phosphosulfate

0014-5793/01/$20.00
PII: S0014-5793(01)02358-4

concentration of 0.5 mM. The cells were further cultured for 2 h, and then harvested.

2.3. Preparation of yeast membrane vesicles

Membrane vesicles were prepared essentially as previously described [8]. Cells were washed with ice-cold 10 mM NaN_3 and converted into spheroplasts in a spheroplast solution containing 1.4 M sorbitol, 50 mM potassium phosphate (pH 7.5), 10 mM NaN_3, 0.25% (v/v) 2-mercaptoethanol, and 2 mg of zymolyase-100T (Seikagaku, Tokyo) per g of cells, and incubated at 37°C for 20 min. The spheroplasts were pelleted, resuspended in five volumes of lysis buffer containing 0.8 M sorbitol, 10 mM HEPES–Tris (pH 7.4), 1 mM EDTA and a protease-inhibitor cocktail (Complete, EDTA-free, Roche Diagnostics), and homogenized using a Teflon homogenizer. The lysate was centrifuged at $1500\times g$ for 10 min to remove unlysed cells and debris. The supernatant was centrifuged at $10\,000\times g$ for 10 min to yield a pellet of membrane vesicles, P10, and the supernatant, S10. The S10 was then centrifuged at $100\,000\times g$ for 60 min to yield a pellet of membrane vesicles, P100, and the supernatant, S100. The P10 and P100 fractions were resuspended in the lysis buffer.

2.4. Transport assay

The transport assay was performed essentially as previously described [12]. Briefly, the transport reaction mixture (100 μl) used in assays contained yeast membrane vesicles (usually 50 μg of protein), 1 μM radiolabeled substrate, 0.8 M sorbitol, 10 mM Tris–HCl (pH 7.0),

A

```
-32                                  CCCGCCGCCAGGCCACCGCTGCTGCGGCAGCC    -1
  1 ATGGCGGAAGTTCATAGACGTCAGCATGCTCGGGTTAAAGGAGAAGCCCCCGCGAAATCC    60
  1  M  A  E  V  H  R  R  Q  H  A  R  V  K  G  E  A  P  A  K  S     20

 61 TCCACACTCCGAGATGAGGAGGAGCTGGGGATGGCGTCGGCCGAAACGCTGACCGTGTTT    120
 21  S  T  L  R  D  E  E  E  L  G  M  A  S  A  E  T  L  T  V  F     40

121 CTGAAGCTGCTGGCCGCCGGCTTTTACGGCGTGAGCTCCTTCCTGATCGTGGTGGTGAAT    180
 41  L  K  L  L  A  A  G  F  Y  G  V  S  S  F  L  I  V  V  V  N     60

181 AAGAGCGTGCTCACCAATTACAGATTTCCCTCCTCACTATGTGTTGGACTTGGCCAGATG    240
 61  K  S  V  L  T  N  Y  R  F  P  S  S  L  C  V  G  L  G  Q  M     80

241 GTGGCCACAGTGGCAGTTCTCTGGGTGGGAAAGGCGCTCAGAGTAGTCAAGTTTCCTGAC    300
 81  V  A  T  V  A  V  L  W  V  G  K  A  L  R  V  V  K  F  P  D    100

301 CTTGACAGAAATGTACCTCGAAAGACGTTTCCACTACCTCTACTATATTTTGGGAACCAA    360
101  L  D  R  N  V  P  R  K  T  F  P  L  P  L  L  Y  F  G  N  Q    120

361 ATCACGGGACTGTTCAGCACAAAGAAACTGAACTTGCCAATGTTTACAGTTCTGAGAAGG    420
121  I  T  G  L  F  S  T  K  K  L  N  L  P  M  F  T  V  L  R  R    140

421 TTCTCCATCCTGTTTACAATGTTTGCTGAAGGAGTTTTACTCAAGAAGACTTTTTCTTGG    480
141  F  S  I  L  F  T  M  F  A  E  G  V  L  L  K  K  T  F  S  W    160

481 GGTATTAAAATGACTGTATTTGCAATGATTATTGGAGCCTTTGTAGCTGCCAGCTCTGAC    540
161  G  I  K  M  T  V  F  A  M  I  I  G  A  F  V  A  A  S  S  D    180

541 TTGGCATTTGATCTGGAAGGATATGCTTTTATTCTGATAAACGATGTCCTAACAGCAGCA    600
181  L  A  F  D  L  E  G  Y  A  F  I  L  I  N  D  V  L  T  A  A    200

601 AATGGTGCATACGTAAAACAAAAATTAGATTCAAAAGAGCTGGGAAAATATGGACTGCTC    660
201  N  G  A  Y  V  K  Q  K  L  D  S  K  E  L  G  K  Y  G  L  L    220

661 TATTACAATGCACTGTTCATGATTCTGCCCACCCTGGCCATTGCGTATTTCACAGGAGAT    720
221  Y  Y  N  A  L  F  M  I  L  P  T  L  A  I  A  Y  F  T  G  D    240

721 GCACAAAAGGCTGTGGAGTTTGAAGGCTGGGCTGACACCCTCTTTCTTCTGCAGTTCACC    780
241  A  Q  K  A  V  E  F  E  G  W  A  D  T  L  F  L  L  Q  F  T    260

781 CTCTCCTGTGTGATGGGGTTTATCTTAATGTACGCCACAGTACTCTGCACGCAGTATAAT    840
261  L  S  C  V  M  G  F  I  L  M  Y  A  T  V  L  C  T  Q  Y  N    280

841 TCTGCTCTTACAACTACAATAGTTGGCTGTATTAAGAATATATTAATAACTTATATTGGA    900
281  S  A  L  T  T  T  I  V  G  C  I  K  N  I  L  I  T  Y  I  G    300

901 ATGGTCTTTGGTGGAGATTATATTTTCACGTGGACAAACTTCATTGGTTTAAATATCAGC    960
301  M  V  F  G  G  D  Y  I  F  T  W  T  N  F  I  G  L  N  I  S    320

961 ATTGCTGGGAGCCTGGTATATTCCTATATCACTTTCACTGAAGAGCAGCTGAGCAAACAG   1020
321  I  A  G  S  L  V  Y  S  Y  I  T  F  T  E  E  Q  L  S  K  Q    340

1021 TCAGAGGCTAATAACAAGCTGGACATTAAGGGGAAAGGAGCAGTGTGACCAGAGGATTGC   1080
 341  S  E  A  N  N  K  L  D  I  K  G  K  G  A  V  *                355

1081 TTCATCTGATACGTAGGCCTAAGTTTTTAATCAACTCAGAACACTGGCAGTTCCTACAGA   1140
1141 GACACTGAGGTGTCTTGATTATGGAGGAAATAACCATTCCTTTGCACTTGTACAATCTGA   1200
1201 GGATTGATTGCTGCCTTTTAAAATTTTATGAAGAGAGAAA                       1240
```

Fig. 1. Cloning of the hUGTrel7 (human UDP-GlcA/UDP-GalNAc dual transporter) cDNA. A: The nucleotide sequence and the deduced amino acid sequence of hUGTrel7. The DDBJ/EMBL/GenBank accession number of the nucleotide sequence is AB044343. B: Northern blot analysis. (a) Each membrane (Clontech human multiple tissue Northern blot) was hybridized with a radiolabeled cDNA fragment encoding the entire ORF, which was amplified by PCR from clone 1989970 as described [8]. (b) Hybridization with human β-actin cDNA (Clontech). C: FISH was performed using lymphocytes isolated from human blood and a 1.3-kb cDNA fragment from clone 1989970 as described before [8]. (a) The arrow indicates the positive signal. (b) Summary of superimposition on DAPI banding images. FISH signals and the DAPI banding patterns were recorded separately by making photomicrographs, and the chromosomal location of the gene was determined by superimposing FISH signals with DAPI-banded chromosomes.

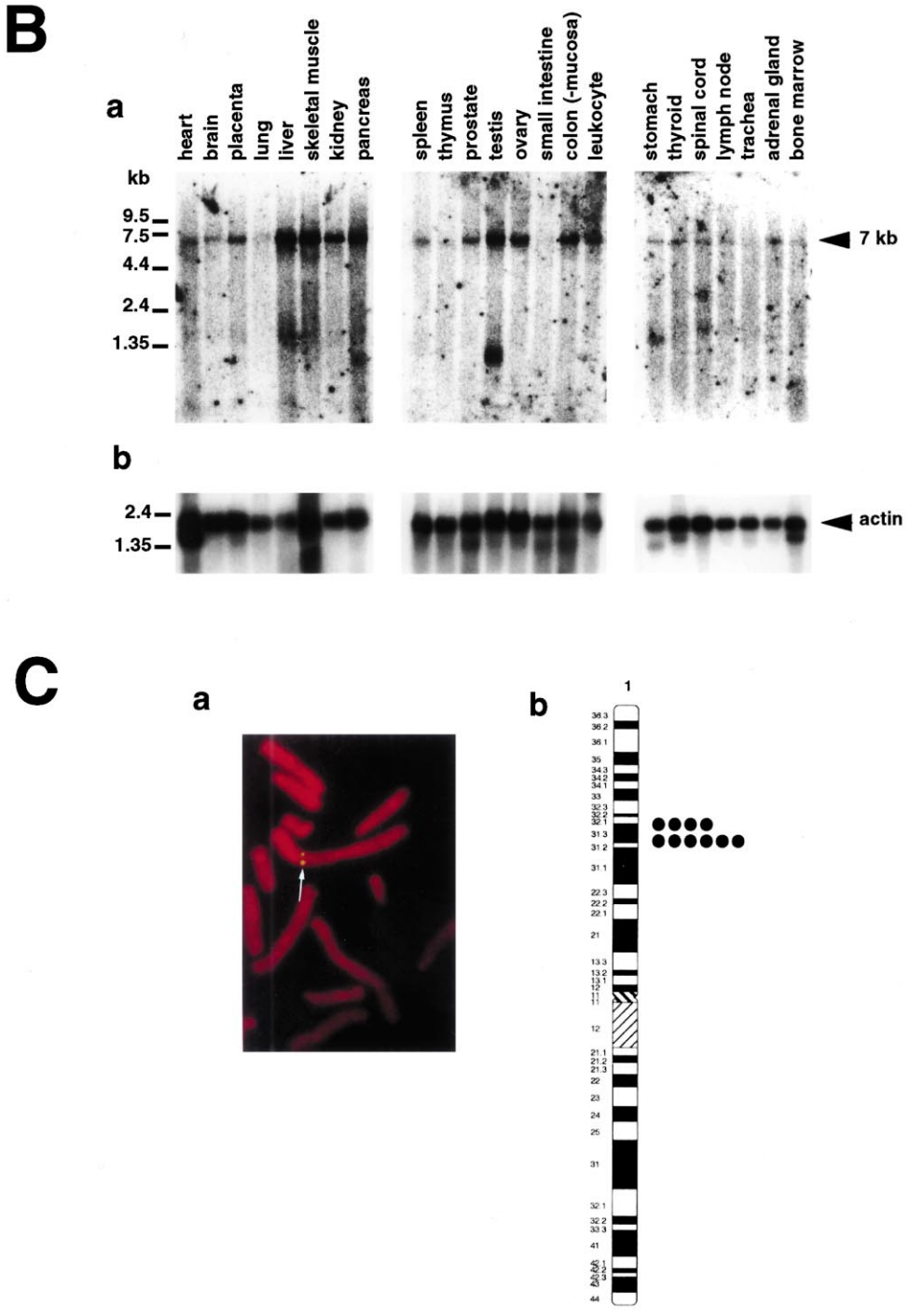

Fig. 1 (*continued*).

2 mM $MgCl_2$ and 0.5 mM dimercaptopropanol. The reaction mixture was incubated at 30°C. The reaction was started by addition of membrane vesicles, and terminated at the appropriate time by an 11-fold dilution with ice-cold stop buffer containing 0.8 M sorbitol, 10 mM Tris–HCl (pH 7.0), 2 mM $MgCl_2$ and 1 μM non-radiolabeled substrate. The entire reaction mixture was passed through a nitrocellulose filter (Millipore). The filter was washed three times with 1 ml of the ice-cold stop buffer, dried, and then the radioactivity trapped on the filter was determined.

2.5. Immunoblotting

Immunoblotting was carried out as described previously [8]. The HA-tagged proteins were detected with rat anti-HA monoclonal antibody 3F10 (Roche Diagnostics).

2.6. Immunofluorescence

The CHO-K1 cells were transfected with an appropriate expression vector using LipofectAMINE reagent (Life Technologies), following the manufacturer's instructions. 24 h after transfection, the cells were transferred onto a Lab-Tek eight-well chamber glass slide (Nalge Nunc International, Naperville, IL, USA) and grown for another 24 h. Then the cells were fixed with 3.7% formaldehyde and permeabilized with 0.1% Triton X-100. The permeabilized cells were stained with rat anti-HA mAb 3F10 followed by fluorescein isothiocyanate-conjugated anti-rat IgG and with either a rabbit polyclonal antibody against α-mannosidase II followed by anti-rabbit IgG-Alexa 546, or a rabbit polyclonal antibody against ERp57 followed by anti-rabbit IgG-Alexa 546. Fluorescence labeling was visualized under a laser scanning confocal microscope (Carl Zeiss LSM510). Antibodies

against α-mannosidase II and ERp57 were kindly provided by Dr. K. Moremen (University of Georgia, Athens, GA, USA) and by Dr. H. Taira (Iwate University, Morioka, Japan), respectively.

3. Results

3.1. Isolation of hUGTrel7 cDNA

We previously identified and characterized several genes closely related to UDP-Gal transporter (UGTrel genes) such as human CMP-Sia transporter [6] and human UDP-GlcNAc transporter [8], through a search of the dbEST. This approach was expanded to identify additional, rather distantly related genes. In this study, we have searched the dbEST for sequences similar to the primary structure of human UGT-related isozyme 1 (hUGTrel1) [9], and found that IMAGE cDNA clone 1989970 encoded a protein that has significant similarity with the hUGTrel1 amino acid sequence. Analysis of the nucleic acid sequence of clone 1989970 revealed that it contains an ORF, which encodes a protein with 355 amino acid residues, as shown in Fig. 1A. We tentatively named this gene human UGTrel7. A database search revealed that clone 1989970 encodes the entire ORF for KIAA0260 protein with unknown function which was reported by Nagase et al. [13]. Expression of hUGTrel7 was ubiquitous and the mRNA was detected as a single major band 7.0 kb in length in Northern blot analysis (Fig. 1B). Fluorescence in situ hybridization (FISH) analysis clearly mapped the hUGTrel7 gene to chromosome 1, p31–p32 (Fig. 1C).

3.2. Subcellular localization of hUGTrel7

hUGTrel7 showed significant similarity with hUGTrel1, a member of the nucleotide sugar transporter subfamily, which is distantly related to the Golgi UDP-Gal transporter [9]. We therefore assumed that hUGTrel7 would also be a transporter protein residing in organellar membranes, and investigated the subcellular localization of hUGTrel7. We first tried to determine the intracellular distribution of the endogenously expressed hUGTrel7 protein using antibodies against the C-terminal segment of the protein, but it was unsuccessful. The amount of the endogenous hUGTrel7 protein may be too small to be detected clearly by this method. The DNA sequence encoding hUGTrel7 tagged with an HA epitope at the C-terminus was then introduced into the mammalian expression vector pMKIT-neo. The construct was transiently expressed in CHO-K1 cells. The cells were doubly immunostained with anti-HA monoclonal antibody and antiserum to either α-mannosidase II or ERp57, marker proteins for Golgi apparatus or endoplasmic reticulum, respectively. The distribution of HA-tagged hUGTrel7 was co-localized with that of ERp57, while it was not co-localized with that of α-mannosidase II (Fig. 2). These data indicate that hUGTrel7 is located in the endoplasmic reticulum in mammalian cells. It should be

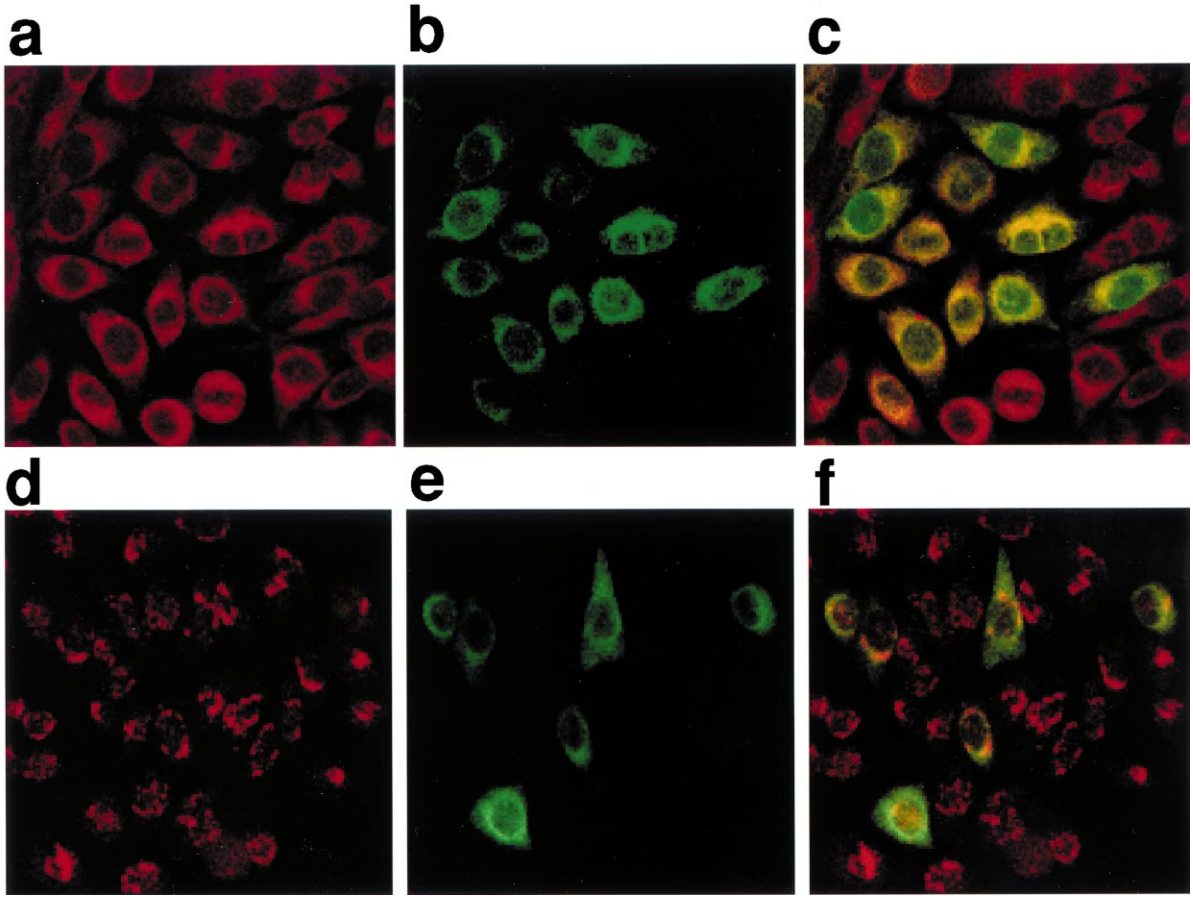

Fig. 2. Subcellular localization of the hUGTrel7 protein. CHO-K1 cells were transfected with pMKIT-neo-hUGTrel7cHA. Forty-eight hours after transfection, cells were fixed and permeabilized with 0.1% Triton X-100. Analysis by indirect immunofluorescence was performed using rabbit anti-ERp57 serum (a), rabbit anti-α-mannosidase II serum (d), and anti-HA rat mAb 3F10 (b, e). The fluorescence images (a) and (b), and (d) and (e), are merged to yield (c) and (f), respectively.

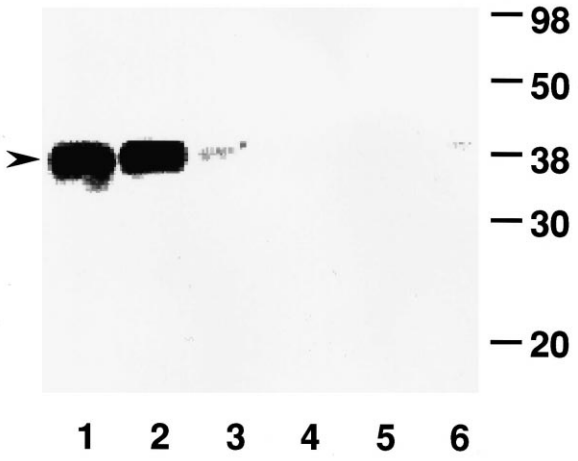

Fig. 3. Expression of the hUGTrel7 protein. P10 (lanes 1, 4), P100 (lanes 2, 5) and S100 (lanes 3, 6) fractions were prepared from yeast carrying the expression vector pYEX-BX with (lanes 1, 2, 3) or without (lanes 4, 5, 6) hUGTrel7cHA. An equivalent amount of each fraction in terms of cell equivalents was analyzed by Western blotting with anti-HA mAb 3F10. The arrowhead indicates hUGTrel7cHA.

noted that C-terminally added HA tags did not disturb the Golgi localization of nucleotide sugar transporters such as CMP-Sia [6] and UDP-GlcNAc [8] transporters.

3.3. Expression of hUGTrel7 in the yeast S. cerevisiae

To test the nucleotide sugar transport activity of hUGTrel7, we utilized a heterologous expression system of hUGTrel7 in *S. cerevisiae*. We showed previously that nucleotide sugar transporters were expressed in active form in *S. cerevisiae* [8,12] and that yeast microsomes have only very low intrinsic nucleotide sugar transporting activity except for a potent GDP-mannose transporting activity.

The DNA sequence encoding hUGTrel7 tagged with an HA epitope at the C-terminus was introduced into the pYEX-BX vector. The plasmids with/without the hUGTrel7 cDNA insert were transfected into *S. cerevisiae* YPH500. The membrane fractions (P10 and P100) and the soluble fraction (S100) were prepared from the transformed cells grown in YNBD medium after induction with 0.5 mM $CuSO_4$. Fig. 3 shows an immunoblot of these fractions probed with anti-HA antibody. The P10 and P100 fractions from the hUGTrel7 transformant contained a 37-kDa HA-tagged protein, whose apparent molecular mass is consistent with the one predicted for HA-tagged hUGTrel7 (40 kDa). This 37-kDa protein was not detected in any fraction from cells transfected with vector alone. These data indicate that the hUGTrel7 was heterologously expressed in *S. cerevisiae* intracellular membranes.

3.4. Identification of hUGTrel7 as a UDP-GlcA/UDP-GalNAc transporter

The uptake of eight nucleotide sugars and PAPS by P10 membrane vesicles expressing hUGTrel7 was examined using a filtration method (Fig. 4). At least five times more UDP-GlcA and UDP-GalNAc was taken up by membrane vesicles from hUGTrel7-expressing cells than by control membrane vesicles from cells not expressing hUGTrel7. Considerably more UDP-GlcNAc was also incorporated into membrane vesicles expressing hUGTrel7 than into those without hUGTrel7 protein. However, we cannot decide at present whether UDP-GlcNAc serves as a substrate for hUGTrel7 protein or not, because of lower but significant uptake of UDP-GlcNAc by control membrane vesicles not expressing hUGTrel7 protein. The less than two-fold difference in incorporation of nucleotide sugar between hUGTrel7-expressing and non-expressing membranes is not large enough to permit a definitive conclusion. We also cannot exclude the possibility that hUGTrel7 transports UDP-Glc and GDP-Man. The endogenous levels of transport of these nucleotide sugars were rather high in *S. cerevisiae*, and might have obscured the transport of these nucleotide sugars by hUGTrel7. Purification and reconstitution of the hUGTrel7 protein will be required for conclusive determination of its substrate specificity spectrum.

The time course of uptake of UDP-GlcA and UDP-GalNAc by membrane vesicles with or without hUGTrel7 was also studied (Fig. 5). In membrane vesicles from hUGTrel7-expressing cells, uptake of the nucleotide sugar initially proceeded rapidly for about 1 min and then proceeded slowly for up to 30 min. On the other hand, the uptake of UDP-GlcA and UDP-GalNAc by the membrane vesicles from vector control cells only exhibited the slow phase throughout the 30 min. These data further confirmed that the hUGTrel7 protein was involved in the uptake of UDP-GlcA and UDP-GalNAc by membrane vesicles.

To determine whether the uptake in fact represented entry of UDP-GlcA and UDP-GalNAc into the lumen of the vesicles, and not simple binding to the hUGTrel7 protein, we measured the nucleotide sugar incorporation with membrane vesicles (P10) permeabilized by treatment with *Staphylococcus aureus* α-toxin. As shown in Fig. 5, permeabilized vesicles were unable to accumulate the nucleotide sugars. These data indicate that uptake of UDP-GlcA and UDP-GalNAc represents the net transport of the substrates into the lumen of the vesicles. Based on these results, we concluded that hUGTrel7 is a UDP-GlcA and UDP-GalNAc transporter.

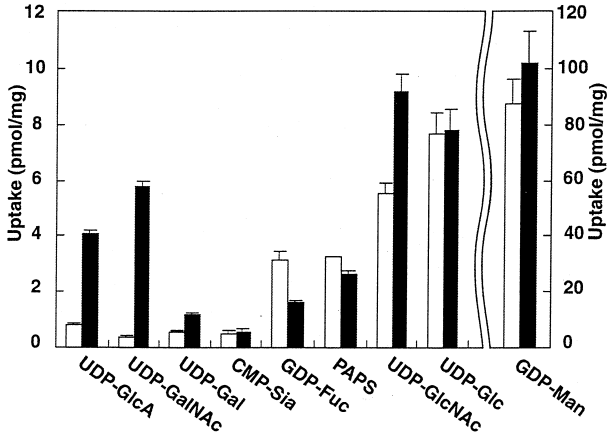

Fig. 4. Substrate specificity of UGTrel7. Membrane vesicles (10 μg of protein for GDP-mannose uptake, 50 μg of protein for the others) from hUGTrel7-expressing *S. cerevisiae* cells (black bars) or cells not expressing hUGTrel7 (white bars) were incubated in 100 μl of the transport reaction mixture containing 1 μM radiolabeled substrate at 30°C for 1 min. The uptake of each substrate by the membrane vesicles was measured by the filtration method described in Section 2. Values are mean ± S.E.M. from duplicate experiments.

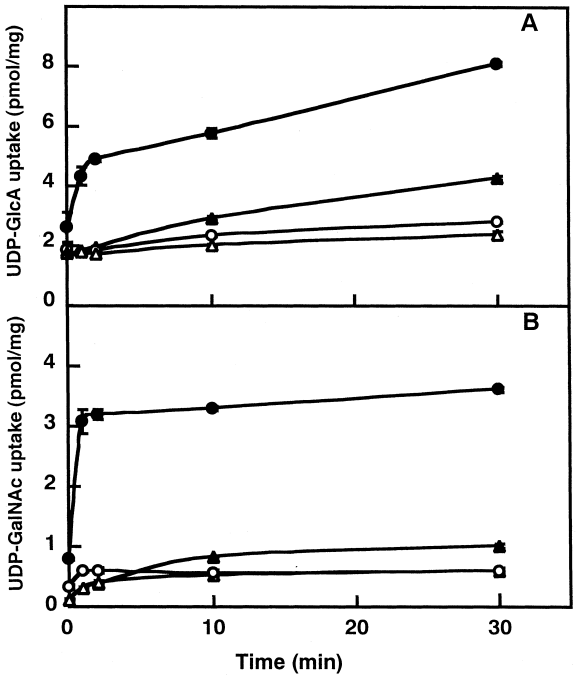

Fig. 5. Effect of *S. aureus* α-toxin on the uptake of UDP-GlcA and UDP-GalNAc by membrane vesicles. Membrane vesicles (P10; 50 μg of protein) from hUGTrel7-expressing *S. cerevisiae* cells (circles) or cells not expressing hUGTrel7 (triangles) were preincubated on ice for 1 h in the absence (solid symbols) or presence (open symbols) of 160 μg of *S. aureus* α-toxin (Sigma)/mg protein. Thereafter, the preincubated membrane vesicles were incubated in 100 μl of the transport reaction mixture containing 1 μM UDP-[^{3}H]GlcA (A) or 1 μM UDP-[^{3}H]GalNAc (B) at 30°C and the uptake of labeled substrates by membrane vesicles was monitored as a function of time. Values are mean ± S.E.M. from duplicate experiments. Note that majority of error bars are hidden by symbols representing data points.

4. Discussion

The data presented here demonstrate that hUGTrel7 transports at least two kinds of nucleotide sugars, namely UDP-GlcA and UDP-GalNAc. This is the first NST in higher eukaryotes that can transport more than one nucleotide sugar. Recently, Puglielli et al. [14] identified a UDP-GalNAc transporter in rat liver Golgi membranes, and purified it from the membranes. It is likely that hUGTrel7 is distinct from this transporter, since the transporter from rat liver lacks the UDP-GlcA transport activity. This leads to the new concept that one nucleotide sugar species may be transported by various kinds of transporter molecules. Attempts to clarify the differences in transport activities and in physiological roles between the two UDP-GalNAc transporters would contribute much to the understanding of possible regulatory roles of nucleotide sugar transporters in cellular glycosylation systems.

Immunofluorescence study demonstrated that HA-tagged hUGTrel7 protein was localized in the ER when HA-tagged hUGTrel7 cDNA was transiently expressed in CHO cells, although we cannot rule out the possibility that the distribution might somehow be perturbed due to its overexpression. It is therefore likely that hUGTrel7 represents the UDP-GlcA transporter whose occurrence in the ER has long been recognized [15,16] and that hUGTrel7 is involved in supplying the substrate nucleotide sugar for glucuronidation, which occurs within the ER lumen [17,18]. On the other hand, the physiological significance of UDP-GalNAc transport activity of hUGTrel7 remains obscure, since UDP-GalNAc transferase activity has so far been detected in the Golgi apparatus but not in the ER. However, earlier works demonstrated that UDP-GalNAc as well as UDP-GlcA was translocated into ER-derived membrane vesicles [15,16,19]. More recently, it was shown that on relocation of chimeric GalNAc transferases to the ER, GalNAc was added to an ER-retained form of CD8 (CD8/E19), suggesting that all components, including UDP-GalNAc, necessary for initiation of *O*-glycosylation are present in the ER except for GalNAc transferases [20]. In view of these observations, hUGTrel7 is a good candidate for the so far unidentified UDP-GalNAc transporter in the ER, although its physiological roles remain to be determined. One possibility is that UDP-GalNAc plays regulatory roles by affecting UDP-GlcA uptake into the ER. A regulatory effect of UDP-GlcNAc on UDP-GlcA transport activity in the ER was suggested by Bossuyt and Blanckaert [21]. By analogy, UDP-GalNAc in the cytosol may compete with UDP-GlcA by acting as a *cis*-inhibitor, and/or UDP-GalNAc accumulated in the ER lumen may serve as a *trans*-stimulator for uptake of UDP-GlcA.

The possible involvement of this dually specific transporter in chondroitin sulfate biosynthesis is an intriguing but still unconfirmed possibility. Chondroitin sulfate synthesis takes place in the Golgi lumen [22]. The UDP-GlcA/UDP-GalNAc transporter (UGTrel7) is expressed in the ER membrane, and may seem unrelated to chondroitin sulfate biosynthesis. As judged by the observed intracellular localization, UDP-GalNAc-specific transporter isolated from rat liver Golgi may be responsible for this process. It should be noted, however, that UGTrel7 can alone provide both nucleotide sugars required for chondroitin sulfate biosynthesis, and its substrate specificity is reminiscent of the dually specific glycosyltransferase involved in chondroitin sulfate biosynthesis, β-*N*-acetylgalactosaminyltransferase II/β-glucuronyltransferase II, which was recently purified by Tsuchida et al. [23]. According to a proposed model [24], sorting of Golgi-resident proteins to their proper destination may be affected and determined by homo- and/or hetero-oligomer formation among partner membrane proteins. If this is the case for the sorting of the UGTrel7 transporter, then the intracellular distribution obtained under conditions of overexpression directed by a strong promoter may not reflect its localization under physiological conditions due to a shortage of its partner, possibly the dually specific GalNAc/GlcA transferase. It is tempting to speculate that dually specific nucleotide sugar transporter and glycosyltransferase behave in concert in chondroitin sulfate biosynthesis, although at present we do not have evidence supporting this speculation. One direct approach to these issues would be a knock-out analysis using mice, fruit flies or nematodes.

Acknowledgements: This work was supported in part by Grant-in-Aid for Scientific Research No. 11480172, Grant-in-Aid for Scientific Research on Priority Area No. 12033222 from the Ministry of Education, Science, Sports and Culture of Japan and a Grant from Mizutani Foundation for Glycoscience.

References

[1] Hirschberg, C.B., Robbins, P.W. and Abeijon, C. (1998) Annu. Rev. Biochem. 67, 49–69.

[2] Kawakita, M., Ishida, N., Miura, N., Sun-Wada, G-H. and Yoshioka, S. (1998) J. Biochem. 123, 777–785.
[3] Miura, N., Ishida, N., Hoshino, M., Yamauchi, M., Hara, T., Ayusawa, D. and Kawakita, M. (1996) J. Biochem. 120, 236–241.
[4] Eckhardt, M., Mühlenhoff, M., Bethe, A. and Gerardy-Schahn, R. (1996) Proc. Natl. Acad. Sci. USA 93, 7572–7576.
[5] Eckhardt, M. and Gerardy-Schahn, R. (1997) Eur. J. Biochem. 248, 187–192.
[6] Ishida, N., Ito, M., Yoshioka, S., Sun-Wada, G-H. and Kawakita, M. (1998) J. Biochem. 124, 171–178.
[7] Guillen, E., Abeijon, C. and Hirschberg, C.B. (1998) Proc. Natl. Acad. Sci. USA 95, 7888–7892.
[8] Ishida, N., Yoshioka, S., Chiba, Y., Takeuchi, M. and Kawakita, M. (1999) J. Biochem. 126, 68–77.
[9] Ishida, N., Miura, N., Yoshioka, S. and Kawakita, M. (1996) J. Biochem. 120, 1074–1078.
[10] Hara, T., Endo, T., Furukawa, K., Kawakita, M. and Kobata, A. (1989) J. Biochem. 106, 236–247.
[11] Adams, A., Gottschling, D.E., Kaiser, C.A. and Stearns, T. (1997) Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[12] Sun-Wada, G-H., Yoshioka, S., Ishida, N. and Kawakita, M. (1998) J. Biochem. 123, 912–917.
[13] Nagase, T., Seki, N., Ishikawa, K., Ohira, M., Kawarabayasi, Y., Ohara, O., Tanaka, A., Kotani, H., Miyajima, N. and Nomura, N. (1996) DNA Res. 3, 321–329.
[14] Puglielli, L., Mandon, E.C., Rancour, D.M., Menon, A.K. and Hirschberg, C.B. (1999) J. Biol. Chem. 274, 4474–4479.
[15] Nuwayhid, N., Glaser, J.H., Johnson, J.C., Conrad, H.E., Hauser, S.C. and Hirschberg, C.B. (1986) J. Biol. Chem. 261, 12936–12941.
[16] Bossuyt, X. and Blanckaert, N. (1994) Biochem. J. 302, 261–269.
[17] Mulder, G.J. (1992) Annu. Rev. Pharmacol. Toxicol. 32, 25–49.
[18] Meech, R. and Mackenzie, P.I. (1997) Clin. Exp. Pharmacol. Physiol. 24, 907–915.
[19] Abeijon, C. and Hirschberg, C.B. (1987) J. Biol. Chem. 262, 4153–4159.
[20] Röttger, S., White, J., Wandall, H.H., Olivo, J-C., Stark, A., Bennett, E.P., Whitehouse, C., Berger, E.G., Clausen, H. and Nilsson, T. (1998) J. Cell Sci. 111, 45–60.
[21] Bossuyt, X. and Blanckaert, N. (1995) Biochem. J. 305, 321–328.
[22] Silbert, J.E. and Sugumaran, G. (1995) Biochim. Biophys. Acta 1241, 371–384.
[23] Tsuchida, K., Lind, T., Kitagawa, H., Lindahl, U., Sugahara, K. and Lidholt, K. (1999) Eur. J. Biochem. 264, 461–467.
[24] Füllekrug, J. and Nilsson, T. (1998) Biochim. Biophys. Acta 1404, 77–84.