A Single *Caenorhabditis elegans* Golgi Apparatus-Type Transporter of UDP-Glucose, UDP-Galactose, UDP-*N*-Acetylglucosamine, and UDP-*N*-Acetylgalactosamine†

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ABSTRACT: The genome of *Caenorhabditis elegans* encodes for 18 putative nucleotide sugar transporters even though its glycome only contains 7 different monosaccharides. To understand the biological significance of this phenomenon, we have begun a systematic substrate characterization of the above putative transporters and have determined that the gene ZK896.9 encodes a Golgi apparatus transporter for UDP-glucose, UDP-galactose, UDP-*N*-acetylglucosamine, and UDP-*N*-acetylgalactosamine. This is the first tetrasubstrate nucleotide sugar transporter characterized for any organism and is also the first nonplant transporter for UDP-glucose. Evidence for the above substrate specificity and substrate transport saturation kinetics was obtained by expression of ZK896.9 in *Saccharomyces cerevisiae* followed by Golgi enriched vesicle isolation and assays *in vitro*. Further evidence for UDP-glucose transport was obtained by expression of ZK 896.9 in *Giardia lamblia*, an organism recently characterized as having endogenous transport activity for only UDP-*N*-acetylglucosamine. Expression of ZK896.9 was also able to correct the phenotype of a mutant Chinese ovary cell line specifically defective in the transport of UDP-galactose into the Golgi apparatus and of a mutant of the yeast *Kluyveromyces lactis* specifically defective in the transport of UDP-*N*-acetylglucosamine into its Golgi apparatus. Because up to now all three other characterized nucleotide sugar transporters of *C. elegans* have been found to transport two or three substrates, the substrate specificity of ZK896.9 raises questions as to the evolutionary ancestry of this group of proteins in this nematode.

Nucleotide sugar transporters are integral proteins in the endoplasmic reticulum and Golgi apparatus membrane of all eukaryotes studied to date. Their role is to translocate nucleotide sugars from the cytosol, where most are synthesized, into the lumen of the above organelles. Within the lumen, the sugar moiety is the substrate for covalent glycosylation of proteins, proteoglycans, and glycosphingolipids in reactions catalyzed by the corresponding glycosyltransferases. Because approximately 80% of all secreted and membrane-bound proteins in eukaryotes are glycosylated, including such molecules as receptors, hormones, immunoglobulins, and so forth, the above transporters play a crucial role in the biosynthesis and likely the function of these very diverse glycoconjugates (*1–3*). Mutations in the above transporters of the Golgi apparatus result in morphological phenotypes and have been described for humans (*4, 5*), cattle (*6*), *Drosophila* (*7, 8*), *Leishmania* (*9*), and *C. elegans* (*10–12*).

Transporters for nucleotide sugars of the Golgi apparatus are very hydrophobic, multitransmembrane-spanning proteins. For some of them, it was determined that they function as homodimers within the Golgi apparatus membrane (*1, 13–16*). Many of these transporters have significant amino acid sequence identity even though their substrate specificity is rather varied. In addition, transporters with limited sequence identity may have the same substrate specificity. Therefore, one is not able to predict substrate specificity from their primary sequence (*17*). The first transporters that were characterized were highly specific for the translocation of only one substrate. Recent studies have described many examples of these transporters that, although specific, are able to translocate several nucleotide sugars. Translocation of some of these multiple substrates occurs by a competitive transport mechanism, while recent studies have also shown examples where different nucleotide sugars may be translocated simultaneously and independently of each other (*10, 18*).

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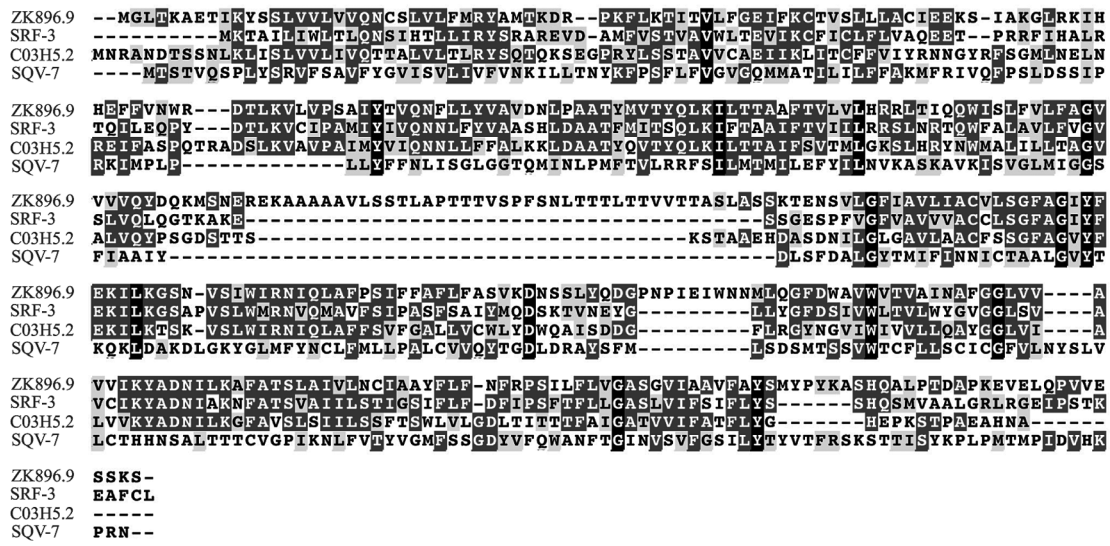

FIGURE 1: Amino acid alignment of nucleotide sugar transporters *C. elegans* ZK896.9 (CAB05326), SQV-7 (NP 495436), CO3H5.2 (NP 493723), and SRF-3 (NP 502447). The Clustal W method was used. Identical residues are highlighted in black, while conservative amino acid substitutions are in gray. As shown, SQV-7 is only 14% identical to CO3H5.2 and 13% identical to SRF-3, although it shares a substrate in common with each of the other two transporters.

The nematode *C. elegans* has multisubstrate nucleotide sugar transporters with overlapping substrate specificities (*3*). Although only seven different sugars occur in its glycoconjugates, the genome encodes 18 putative nucleotide sugar transporters, raising the question of functional redundancy among these transporters. A similar situation occurs in humans (*19*), making this phenomenon a more general and relevant one.

We are using *C. elegans* as a model to study nucleotide sugar transporters, their substrate specificity, and their possible functional redundancy. In *C. elegans*, each of the three nucleotide sugar transporters studied to date are expressed in specific sets of cells (*11, 20*). Several instances of partial overlap in location as well as substrate specificity of nucleotide sugar transporters have been reported along with functional redundancy among such transporters (*12*). To date, the substrate specificity of three *C. elegans* nucleotide sugar transporters has been experimentally determined: SQV-7 transports UDP-Gal[1], UDP-GalNAc, and UDP-GlcA (*10*); SRF-3 transports UDP-Gal and UDP-GlcNAc (*11*), while CO3H5.2, the most recently characterized one, transports UDP-GlcNAc and UDP-GalNAc (*18*).

We here report the substrate specificity of *C. elegans* transporter ZK896.9, a novel tetrasubstrate transporter that translocates UDP-Gal, UDP-GlcNAc, UDP-Glc, and UDP-GalNAc. This substrate specificity was determined by several approaches: (a) demonstration of saturable transport for each of the above substrates into Golgi apparatus-enriched vesicles from *S. cerevisiae* expressing the ZK896.9 transporter; (b) correction of phenotypic mutants of Chinese hamster ovary cells and the yeast *K. lactis* defective in UDP-Gal and UDP-GlcNAc transport, respectively; and (c) heterologous expression in *Giardia lamblia* and demonstration of saturable transport of UDP-Glc. This novel approach takes advantage of *Giardia lamblia* having only one endogenous nucleotide sugar transporter, that for UDP-GlcNAc.

EXPERIMENTAL PROCEDURES

Strain Maintenance and Genetics. S. cerevisiae strain PRY225 (ura3-52, lys2-801am, ade2-1020c, his3, leu2, trp1-1Δa) was grown at 30 °C in liquid yeast extract/peptone/dextrose or on solid yeast extract/peptone/dextrose media containing 2% Bacto-agar. Strains derived from PRY225 transformed with URA plasmids were grown at 30 °C in synthetic complete medium lacking uracil prepared by using SCM-URA (Sigma). For transformation with pG426 or pG426-ZK896.9-VSV, a lithium acetate/polyethylene glycol method was used (*21*). The following *K. lactis* strains were used: KL3 (Mat a, uraA mnn2-2, arg K+, pKD1+) (*22*) and MW103-1C (Mat a, uraA lysA, arg K+, pKD1+) (*23*). *K. lactis* was grown at 30 °C in synthetic complete medium lacking uracil prepared by using SCM-URA (Sigma).

Chinese hamster ovary cell lines Lec2 (ATTC CRL 1736) (*24*) and Lec8 (ATCC CRL 1737) (*25*) were maintained in alpha medium (Biochrome, Berlin, Germany) supplemented with 10% fetal calf serum. *Giardia lamblia* strain MR4 or WB1267 trophozoites were grown in TYI-S-33 medium supplemented with 10% bovine serum (Biosource) and bovine bile (Sigma Aldrich) at 37 °C.

Molecular Biology. Standard molecular biology protocols were used as described by Sambroock et al. (*26*) unless otherwise noted. The *C. elegans* ZK896.9 cDNA (ORF3 in ref *29*) was amplified by PCR using BIO-X-ACT Short Mix DNA polymerase (BIOLINE) using ZK896.9-specific primers. The cDNA was fused in frame with a single VSV-G tag coding sequence by PCR mutagenesis and cloned into pG426 for expression in *S. cerevisiae*, into pE4 for expression in *K. lactis*, and into the *G. lamblia* pGDP-2*Bgl*II vector.

Radioactive Substrates. The following radioactive substrates used were all purchased from American Radiolabeled Chemicals: UDP-[^{3}H]Glc (60 Ci/mmol), UDP-[^{3}H]Gal (20 Ci/mmol), UDP-[^{3}H]GalNAc (60 Ci/mmol), and UDP-[^{3}H]GlcNAc (60 mCi/mmol). UDP-[^{14}C]-Xyl (264 mCi/

[1] Abbreviations: UDP-Gal, Uridine diphosphate galactose; UDP-GlcNAc, uridine diphosphate *N*-acetylglucosamine; UDP-Glc, uridine diphosphate glucose; UDP-GalNAc, uridine diphosphate *N*-acetylgalactosamine; CHO, Chinese hamster ovary.

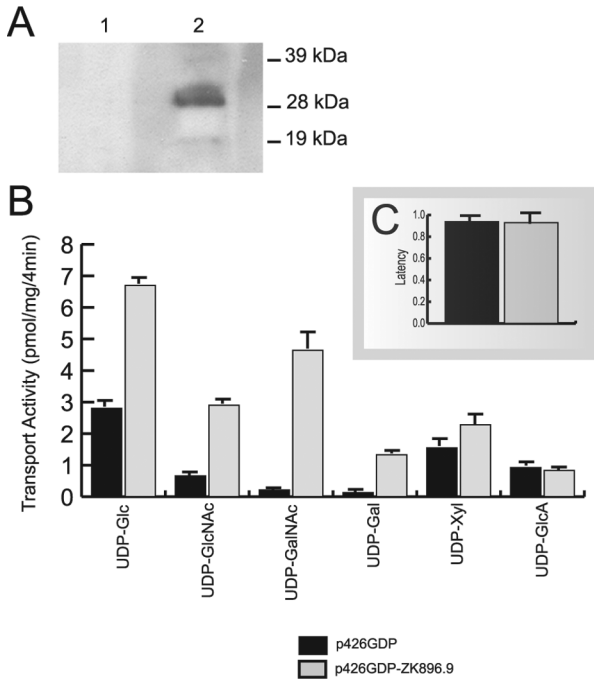

FIGURE 2: Functional expression of VSV-tagged ZK896.9 in *S. cerevisiae*. (A) Western blot analysis. Twenty-five micrograms of membrane protein from *S. cerevisiae* transformed with the empty vector (lane 1) or the vector encoding ZK896.9-VSV (lane 2) was applied to each lane of a 4–12% SDS−polyacrylamide gel and subjected to immunoblot analysis with anti-VSV-monoclonal antibody as described in Experimental Procedures. (B) Nucleotide sugar transport into *S. cerevisiae* Golgi enriched vesicles. White bars, vesicles from cells transformed with empty vector; black bars, vesicles from cell transformed with ZK896.9-VSV. Vesicles were isolated as described in Experimental Procedures and assayed for their ability to transport different nucleotide sugars. The transport activity shown is the amount of nucleotide sugars inside vesicles at 25 °C minus the corresponding amount at 0 °C. The concentration of nucleotide sugars in the reaction medium was 2 μM. The results shown are the average of two determinations. The error bars represent standard error. (C) Latency of GDPase activity. Intact vesicles or vesicles pretreated with 0.1% Triton-X100 were used to assay for GDP/UDPase activity.

mmol) and UDP-[^{3}H]GlcA (15 Ci/mmol) were purchased from Perkin-Elmer.

Western Blot Analysis. Total membrane fractions, from 3 mL of liquid yeast cultures (A_{600} = 3.0), were prepared by glass bead disruption of the cells in membrane buffer [0.8 M sorbitol/ 1 mM EDTA/ protease inhibitor tablet (Roche Applied Science) and 10 mM triethanolamine/ acetic acid at pH 7.2 at 4 °C] plus 2 mM DTT. After centrifugation at 134,000*g* for 1 h, total membranes were resuspended in membrane buffer, and proteins were electrophoresed on NuPAGE 4–12% Bis-Tris gels (Invitrogen) and subsequently electrotransferred onto Immobilon-P membranes (Millipore). Nonspecific binding was blocked by incubating the membranes for 1 h at room temperature in 5% nonfat dry milk in phosphate saline buffer. Membranes were incubated for 1 h with mouse anti-VSV (Roche) antibody in phosphate saline buffer. Protein detection was performed by using horseradish peroxidase-conjugated mouse IgG (Promega) followed by chemiluminescence using ECLP Western blotting detection reagents (American Biosciences).

Subcellular Fractionation. S. cerevisiae transformed with pG426 or pG426-ZK896.9-VSV was grown in SCM-URA liquid medium to A_{600} = 3. The culture was centrifuged and converted to spheroplasts as previously described by using a total of 1 mg of Zymolyase 100T (Seigakaku America) per gram of cells. The spheroplast suspension was centrifuged at 2,000*g* for 10 min. Cells were broken by suspending the pellet in 1.5× volume of membrane buffer and by drawing the cell pellet rapidly several times into a narrow-bore serological pipet. The suspension was centrifuged successively for 10 min at 2,000*g*, for 8 min at 5,000*g*, and finally for 45 min at 125,000*g* to obtain a pellet fraction enriched in endoplasmic reticulum (5000*g*)- and Golgi apparatus (125,000*g*)-derived vesicles (*10*).

Giardia lamblia crude membranes were prepared by a modification of the procedure described for COS-1 cells by Enyedi et al. (*27*). Log-phase *Giardia* was chilled on ice for 20 min, collected by centrifugation, and washed twice in PBS. *Giardia* was resuspended in 0.5 mL of hypotonic lysis buffer (10 mM Hepes-KOH at pH 7.2, 10 mM $MgCl_2$, 25 mM KCl, and complete protease inhibitor (Sigma)). After 10 min on ice, cells were homogenized with 40 strokes in a Dounce homogenizer. The homogenate was diluted with an equal volume of the lysis buffer containing 0.5 M sucrose and further homogenized with 25 strokes. The homogenate was centrifuged at 2000*g* for 5 min to remove unbroken cells, and the suspension was centrifuged for 8 min at 5000*g*. The pellet was resuspended in a solution containing 0.5 M sucrose, 10 mM $MgCl_2$, 25 mM KCl, 10 mM Hepes-KOH at pH 7.2, and complete protease inhibitor mixture. Vesicles were used either immediately or frozen at −80 °C in small aliquots.

Nucleotide Sugar Transport Assay. The theoretical basis for the translocation assay of nucleotide sugars into Golgi apparatus-enriched vesicles has been described previously (*2, 3*). Transport assays of *C. elegans* nucleotide sugar transporter expressed in *S. cerevisiae* and analyses of the samples was carried out as described (*18*). Radioactivity was detected by using a liquid scintillation spectrometer. Transport into *Giardia lamblia* vesicles was done as described for *S. cerevisiae* vesicles with the difference that it was done for 5 min at 37 °C.

Nucleotide Sugar-Dependent Transferase Activities in G. lamblia Membranes. Measurements were carried out in Eppendorf tubes using the corresponding radioactive nucleotide sugar plus different concentrations of nonradioactive nucleotide sugar as substrate. The reaction mixture in a final volume of 200 μL contained 10 mM divalent cation ($MnCl_2$, $MgCl_2$, or $CaCl_2$) or EDTA, 0.5 M sucrose, 30 mM triethanolamine at pH 7.2, 25–50 μg of membrane protein (P5K or P130K fraction), and 0.1% Triton X-100.

Nucleotidase Activity. The assay was essentially as described previously (*28*). Briefly, incubation mixtures contained, in a final volume of 100 μL, 10 μg of Golgi apparatus-enriched vesicles, 2 mM $CaCl_2$ or EDTA, 200 mM imidazole at pH 7.5, 2 mM nucleosides diphosphate, and with or without 0.1% Triton X-100. After incubation at 30 °C for 30 min, the reaction was stopped by adding 200 μL of 7.5% SDS. Inorganic phosphate released was determined by adding 700 μL of Ames reagent (0.42% ammonium molybdate in 1 N sulfuric acid/10% ascorbic acid, 6:1), followed by incubation for 20 min at 45 °C. Absorbance was measured at 660 nm.

Complementation of Chinese Hamster Ovary cells. Complementation experiments were carried out by transfecting

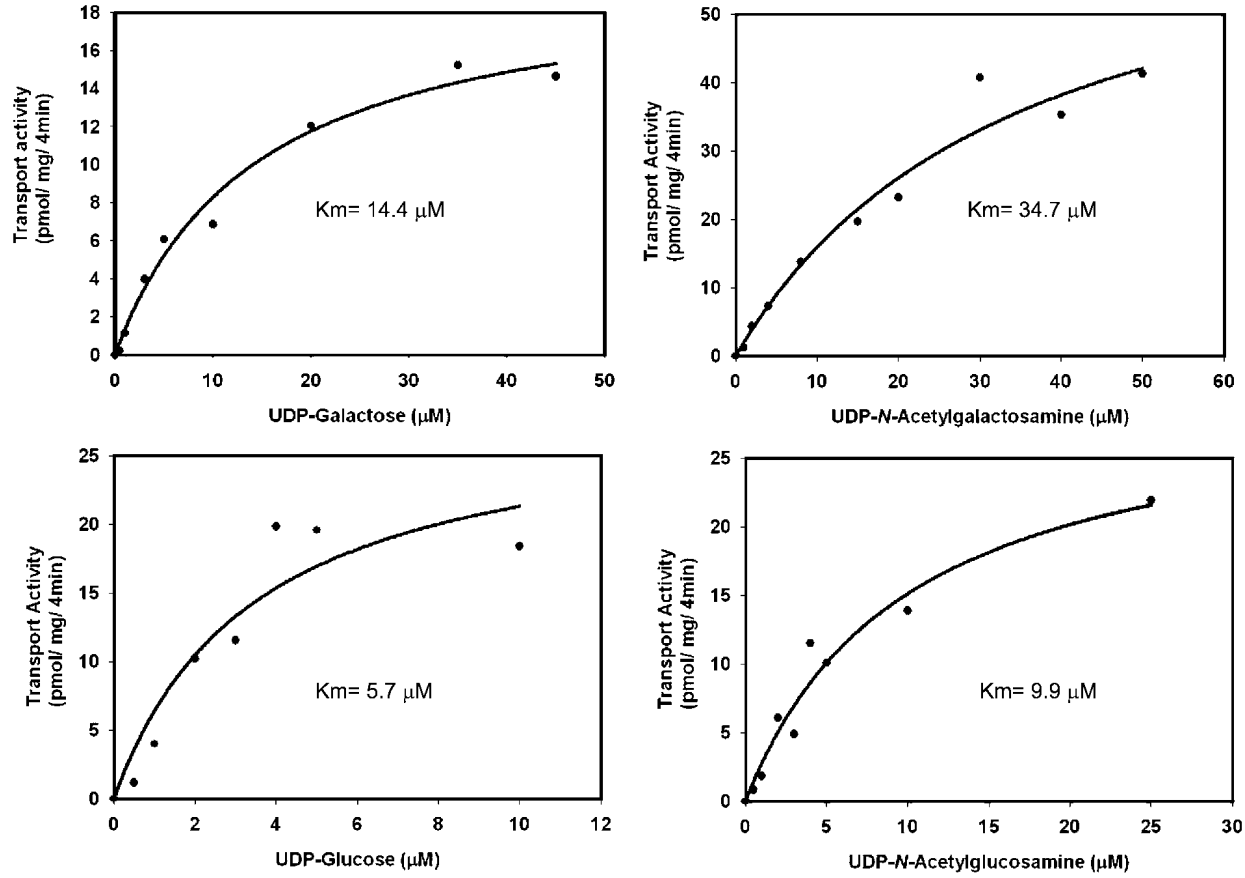

FIGURE 3: Kinetics of nucleotide sugar transport into Golgi apparatus-enriched vesicles from *S. cerevisiae* transformed with ZK896.9-VSV. Vesicles were incubated with different concentrations of radiolabeled nucleotide sugars at 25 °C for 4 min. The line represents the best fit of the data given by the Michaelis–Menten equation using Sigma Plot for Windows. The data points are the average of two experiments with two different vesicle preparations.

2×10^5 cells using Metafectene (Biontex, Munich Germany) with plasmid carrying *C. elegans* ZK896.9 (described as ORF-3 (*29*), mouse CMP-sialic acid transporter (pFLAG-WT) described in ref *15*, and human UDP-Gal transporter (hUGT) described in ref *30*. Two days post transfection, cells were released from the plates by incubation with PBS/2 mM EDTA, pelleted, and taken up in 0.1 mL of lysis buffer (50 mM Tris-HCl at pH 8.0, 150 mM NaCl, 5 mM EDTA, 1% NP-40, 1 mM PMSF, and 100 U/mL Aprotein). Following a 15 min incubation and a 2 min centrifugation at 4 °C, 25 μL of Laemmli buffer was added to the supernate and 25 μL subjected to 10% SDS−PAGE followed by Western blotting. Blots were incubated with antibody 745 (*31*) against polysialic acid, followed by alkaline phosphatase conjugated Goat anti mouse secondary antibody (Jackson Immuno Research), and developed with NBT/BCIP.

RESULTS

Previous studies from our laboratory had shown that the substrate specificity of Golgi apparatus nucleotide sugar transporters cannot be deduced from the protein sequence alone but has to be determined experimentally. This point is illustrated in Figure 1, which shows the amino acid sequences of the three nucleotide sugar transporters from *C. elegans*, which have been previously characterized, and of ZK896.9. Each of them translocates a different but partially overlapping set of substrates. SQV-7 has a very low amino acid sequence identity with SRF-3 (13%) and CO3H5.2 (14%), though it shares the transport of UDP-Gal with SRF-3 and UDP-GalNAc with CO3H5.2. However, SRF-3 and CO3H5.2 have high amino acid sequence identity among them (43%) but only share the translocation of one substrate, UDP-GlcNAc. ZK896.9 encodes 1 of 12 putative nucleotide sugar transporters that had been originally cloned in a project that led to the identification of the first GDP-fucose transporter (*29*). The amino acid sequence of ZK896.9 shares 43 and 40% identity with SRF-3 and C03H5.2, respectively. However, on the basis of the above sequence-function problems, the substrate specificity of the putative transporter ZK896.9 must be determined experimentally.

Putative C. elegans Nucleotide Sugar Transporter ZK896.9 Translocates UDP-glucose, UDP-galactose, UDP-N-acetylgalactosamine, and UDP-N-acetylglucosamine. In order to determine whether the putative transporter ZK896.9 is indeed a nucleotide sugar transporter and if so which substrates it translocates, Golgi apparatus-enriched vesicles from *S. cerevisiae* expressing ZK896.9 fused to a C-terminal VSV epitope (ZK896.9-VSV) were prepared. Figure 2A shows the ZK896.9-VSV-encoded protein expression in the Golgi apparatus-enriched fraction of *S. cerevisiae* transformed with the ZK896.9-VSV encoding vector; no protein is detected in the lane corresponding to the control transformed with the empty vector.

Vesicles prepared as described above were then assayed, in a preliminary manner, for their ability to transport different radiolabeled nucleotide sugars. As can be seen in Figure 2B,

vesicles expressing ZK896.9-VSV showed significantly higher transport activities, compared to that of controls, for UDP-Glc, UDP-GalNAc, UDP-Glc-NAc, and UDP-Gal. No significant transport activity, above that of controls, was detected for UDP-xylose and UDP-glucuronic acid. In a separate experiment, no transport, above that of controls, was detected for GDP-fucose, CMP-sialic acid and GDP-mannose (not shown). As a control of the integrity and topography of the vesicles used in the above assays, we measured the latency of GDPase, a luminal Golgi apparatus activity previously shown to be required for the generation of GMP, the antiporter molecule necessary for the transport of GDP-mannose into *S. cerevisiae* Golgi apparatus vesicles. As can be seen in Figure 2C, vesicles were 90–95% latent toward GDPase, regardless of whether they were derived from yeast transformed with the empty plasmid or that encoding ZK896.9-VSV. Thus, these Golgi apparatus-enriched vesicles were sealed and of the same topographical orientation as that *in vivo*, suggesting that the differences in transport observed are the result of the activity of transporter ZK896.9 in these vesicles.

Strong evidence supporting that the above four substrates were indeed translocated by transporter ZK896.9 was obtained by analyzing the kinetics of transport of the four nucleotide sugars. As shown in Figure 3, transport of each of these four substrates was saturable with apparent *K*m values of 5.7 μM for UDP-Glc, 14.4 μM for UDP-Gal, 34.7 μM for UDP-GalNAc, and 9.9 μM for UDP-GlcNAc. These values are in agreement with the same parameters for other nucleotide sugar transporters of *C. elegans* as well as those from other organisms previously reported (*8, 11*). These experiments therefore strongly suggest that putative *C. elegans* transporter ZK896.9 translocates UDP-Glc, UDP-Gal, UDP-GlcNAc, and UDP-GalNAc *in vitro.*

In previous studies, we had demonstrated that *S. cerevisiae* has endogenous transport activities for GDP-mannose and UDP-Glc (*32, 33*). This makes it difficult to conclusively demonstrate UDP-Glc transport activity by a putative transporter expressed in *S. cerevisiae* or other yeasts such as *S. pombe* or *K. lactis*. It was therefore of great interest to attempt the functional expression of transporter ZK896.9 in an organism with very low or no endogenous UDP-Glc transport activity.

Giardia lamblia has recently been shown to have UDP-GlcNAc as the sole endogenous nucleotide sugar transport activity (Samuelson, J., unpublished results). This observation makes it an attractive system to determine whether or not transporter ZK896.9 has UDP-Glc transport activity. Thus, *G. lamblia* was transformed with vector pGFP-2BgIII alone or encoding *C. elegans* transporter ZK896.9-VSV. As can be seen in Figure 4A, a Western blot analysis shows a protein band in the 45 kDa region (lane 2) in membranes derived from *G. lamblia* transformed with ZK896.9-VSV, which is absent in the protein profile of membranes transformed with the vector without the insert (lane 1). We do not know the reason for the apparent size difference in ZK896.9 when expressed in *S. cerevisiae* and *G. lamblia.*

Following the above results, we determined, in a preliminary experiment, whether transport of UDP-Glc into *G. lamblia*-derived vesicles was dependent on the expression of ZK896.9 and on temperature. As can be seen in Figure 4B, transport of UDP-Glc was dependent on both of the

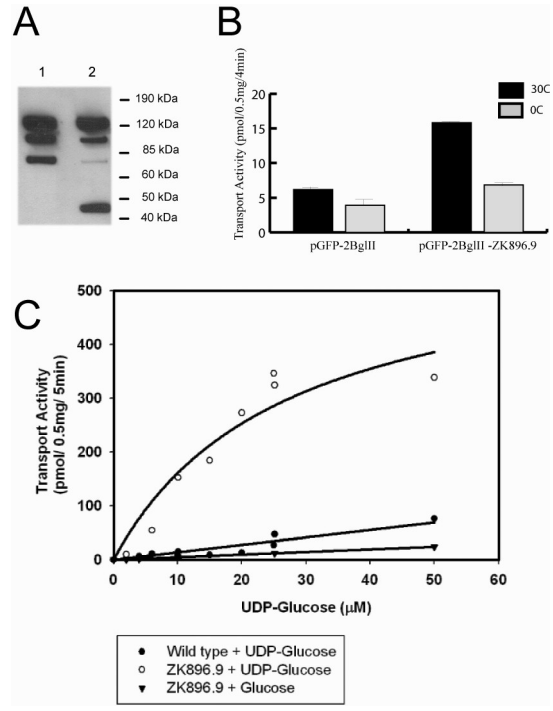

FIGURE 4: Transport of UDP-glucose into vesicles from *Giardia lamblia.* (A) Western blot analysis. Twenty-five micrograms of membrane protein from *Giardia lamblia* transformed with the vector encoding ZK896.9-VSV (lane 2) or the empty vector (lane 1) was applied to 4–12% SDS−polyacrylamide gel electrophoresis and subjected to immunoblot analysis with anti-VSV monoclonal antibody as described in Experimental Procedures. (B) Transport of UDP-Glc. Transport was measured at 0 and 30 °C into vesicles from *G. lamblia* transformed either with the vector encoding ZK896.9-VSV (two right columns) or the empty vector (two left columns). Incubations were for 4 min using 2 μM UDP-Glc. The results are the average of two independent determinations. Error bars represent standard error. (C) Kinetics of UDP-Glc transport. Transport was measured into vesicles from *G. lamblia* transformed with empty vector (●) or with vector encoding ZK896.9-VSV (○). Incubations were for 4 min at 25 °C. The line represents the best fit of the data given by the Michaelis–Menten equation using Sigma Plot for Windows. Vesicles encoding ZK896.9-VSV were also incubated with different concentrations of [^{3}H]Glc (▼) as control.

above parameters. We next determined whether transport of UDP-Glc into *G. lamblia*-enriched vesicles was saturable. As shown in Figure 4C, this was indeed the case with an approximate *K*m of 9.7 μM. This figure also shows that the transport of UDP-Glc into vesicles from *G. lamblia* transformed with the vector alone showed virtually no transport. As an additional control, we also determined that transporter ZK896.9 does not transport free glucose (Figure 4C).

It was important to obtain evidence that some of the substrate specificities previously established *in vitro* also reflect specificities *in vivo.* For this reason, we measured the ability of transporter ZK896.9 to correct the phenotypes of mutants previously shown to be defective in some of the transport activities assigned to transporter ZK896.9. The *K. lactis* mutant mnn-3 is defective in Golgi apparatus transport of UDP-GlcNAc. As a result of a decrease in surface terminal *N*-acetylglucosamine, this mutant binds significantly less of the lectin from *Griffonia simplicifolia* II than the wild type (*17*).

When *K. lactis* mnn-3 mutant cells were transformed with a plasmid encoding the ZK896.9 transporter, binding of the

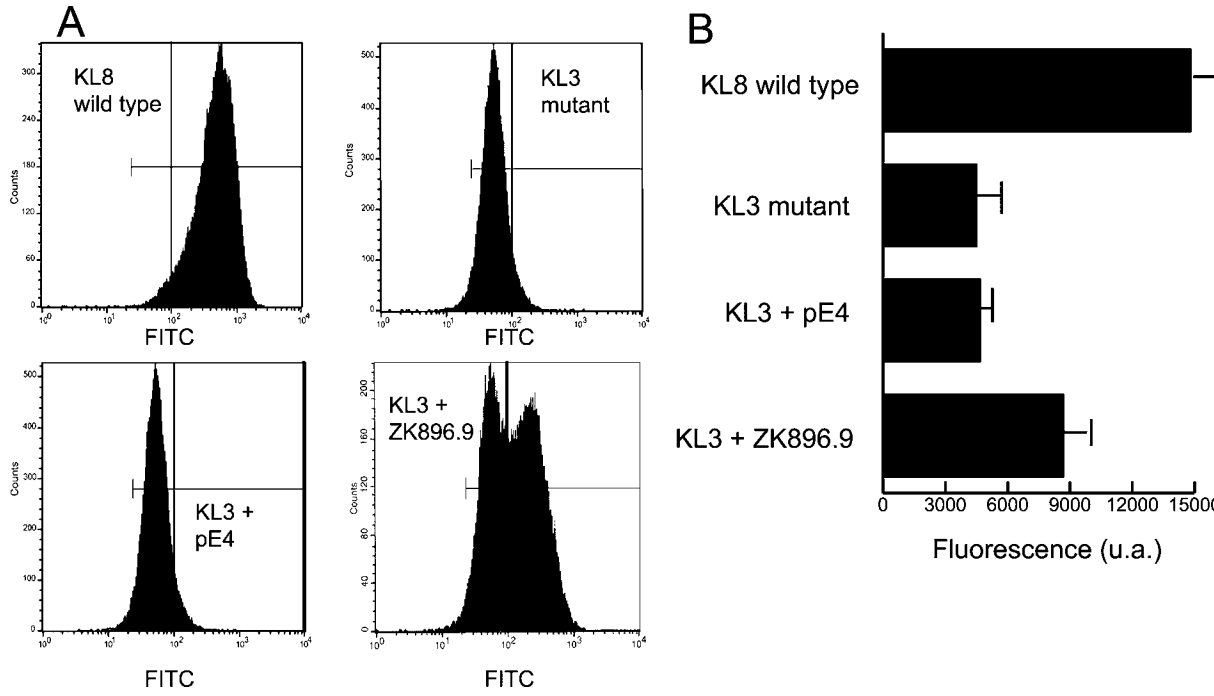

FIGURE 5: Separation of *K. lactis* cells by FACS following cell surface labeling with fluorescent lectin from *Griffonia simplicifolia II*. KL3 mutants, defective in the Golgi apparatus transporter of UDP-GlcNAc, were transformed with the vector pE4 or the vector encoding ZK896.9. Cells were then incubated with the fluorescent lectin and separated by FACS. A: Fluorimetric determination of cell surface labeling. B: Histogram from FACS.

fluorescent lectin was significantly higher than that of the untransformed mutant (Figure 5A, histogram). A significant population of the ZK896.9 expressing cells bound FITC-GSII in a manner similar to that in wild type cells (Figure 5B). A bimodal distribution of GSII binding of mutant *K. lactis* cells transformed with heterologous nucleotide sugar transporters has been previously observed in our studies (*18*) and probably is the result of the failure of some transformed cells to express a functional transporter. Transformation of mutant *K. lactis* cells with the empty vector did not increase binding of the fluorescent-labeled lectin. Together, these results demonstrate that transporter ZK896.9 can translocate UDP-GlcNAc *in vitro* and *in vivo.*

We had previously shown that CHO mutant Lec8 cells are specifically deficient in UDP-Gal transport and that this resulted in a deficiency in galactose and sialic acid-containing glycoconjugates on the cell surface (*34*). We thus determined whether the UDP-Gal transport activity of transporter ZK896.9 was able to restore the wild type phenotype of CHO mutant Lec8 cells. Accordingly, these cells were transformed with a plasmid encoding ZK 896.9 as well as wild type human UDP-Gal and mouse CMP-sialic acid transporters. Restoration of the wild type phenotype was monitored with an antipolysialic acid antibody. Biosynthesis of this polysaccharide depends on prior galactosylation of *N*-glycans of the neural cell adhesion molecule and therefore on the presence of UDP-galactose and CMP-sialic acid in the Golgi apparatus lumen. Both Lec8 and another CHO mutant Lec2, which is deficient in CMP-sialic acid transport, are thus polysialic acid negative. As can be seen in Figure 6, right panel, restoration of the CHO wild type phenotype was accomplished by transformation of CHO Lec8 mutant with transporter ZK896.9 and the human UDP-Gal transporter but not with the mouse CMP-sialic acid transporter. As a control, transporter ZK896.9 was not able to restore the wild type phenotype of Lec2,

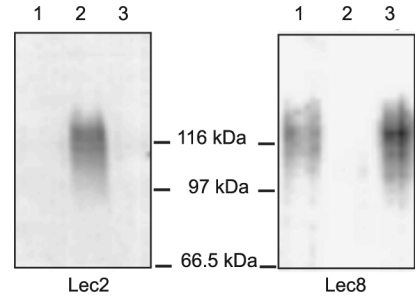

FIGURE 6: Complementation of CHO Lec2 and Lec8 mutants. Lec2 and Lec8 cells, with a defect in CMP-sialic acid and UDP-Gal transport, respectively, were transfected with *C. elegans* ZK896.9 (lane 1), human CMP-sialic acid transporter (lane 2), or human UDP-Gal transporter (lane 3). Complementation was detected by staining of a Western blot with antibody 735, recognizing polysialic acid, for which both Lec8 and Lec2 are negative.

whereas the mouse CMP-sialic acid transporter was able to do so (Figure 6, left panel).

Taken together, the above experiments demonstrate that *C. elegans* nucleotide sugar transporter ZK896.9 translocates UDP-Glc, UDP-Gal, UDP-GlcNAc, and UDP-GalNAc. Translocation of UDP-Glc was also demonstrated to occur into vesicles derived from *G. lamblia* while that of UDP-Gal and UDP-GlcNAc was also demonstrated by *in vivo* correction of mutants deficient in these Golgi apparatus transport activities.

DISCUSSION

We have characterized the substrate specificity of a novel nucleotide sugar transporter, ZK896.9, of the nematode *C. elegans*. Three different approaches were used to demonstrate that this transporter translocates UDP-Gal, UDP-GlcNAc, UDP-Glc, and UDP-GalNAc. The first approach consisted of the expression of ZK896.9 in the yeast *S. cerevisiae*

followed by isolation of Golgi apparatus-enriched vesicles and measurements *in vitro.* All of the above substrates were transported in a saturable manner. Only UDP-Glc is transported endogenously.

In a second approach, we expressed ZK 896.9 in *G. lamblia* followed by isolation of vesicles and measurements of transport *in vitro*. Transport of UDP-Glc had indeed occurred with similar kinetic parameters as previously reported (*11*). As an important control, we also showed that free glucose is not transported into *G. lamblia* vesicles.

The third approach consisted of phenotypic correction of mutant cells previously shown to be defective in the transport of a specific nucleotide sugar. When a mutant of the yeast *K. lactis*, defective in the transport of UDP-GlcNAc (*17*), was transformed with ZK896.9, the mutant regained its surface reactivity to the lectin from *Griffonia simplicifolia* II.

Evidence that transporter ZK896.9 translocates UDP-Gal was also obtained by the transformation of mutant Chinese hamster ovary cells specifically defective in the Golgi apparatus transport of this nucleotide sugar (*34*). These cells regained their ability to bind at their surface an antibody specific for polysialic acid. Because the expression of cell-surface sialic acid depends on the previous incorporation of galactose to surface glycans, this experiment shows that this latter sugar had been incorporated into the glycoconjugates of this mutant cell line.

Is there redundancy between transporter ZK896.9 and other *C. elegans* nucleotide sugar transporters? We have recently found that RNAi of the ZK896.9 gene by feeding wild type and *srf-3* mutant of *C. elegans* does not result in morphological phenotypes (not shown). The latter result is different from what was found with RNAi against *C. elegans* nucleotide sugar transporter CO3H5.2 in *srf-3* mutants (*12*). However, these results do not rule out the redundancy between transporter ZK896.9 and other transporters of this nematode.

Transporter ZK896.9 is the fourth *C. elegans* nucleotide sugar transporter for which the substrate specificity has been determined. While, to our knowledge ZK896.9 is the first nucleotide sugar transporter known to translocate four substrates, all previously characterized nucleotide transporters from this nematode have been found to translocate multiple substrates. Whether transporter ZK 896.9 transports its substrates in a competitive (*10*) or independent and simultaneous manner (*12, 18*) as other previously characterized nucleotide sugar transporters from *C. elegans* remains to be determined.

Transporter ZK896.9 is so far the only *C. elegans* nucleotide sugar transporter that is able to translocate UDP-Glc. This nucleotide sugar is used most likely for the biosynthesis of glucose-containing sphingolipids and other glucose-containing glycoconjugates such as glucose-substituted mucin type *O*-glycans among others, known to occur in *C. elegans* (*35*).

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