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Biochemistry

Expression cloning of the Golgi CMP-sialic acid transporter

(complementation cloning/Lec2 mutant/nucleotide sugar transport/polysialic acid)

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ABSTRACT **Translocation of nucleotide sugars across the membrane of the Golgi apparatus is a prerequisite for the synthesis of complex carbohydrate structures. While specific transport systems for different nucleotide sugars have been identified biochemically in isolated microsomes and Golgi vesicles, none of these transport proteins has been characterized at the molecular level. Chinese hamster ovary (CHO) mutants of the complementation group Lec2 exhibit a strong reduction in sialylation of glycoproteins and glycolipids due to a defect in the CMP-sialic acid transport system. By complementation cloning in the mutant 6B2, belonging to the Lec2 complementation group, we were able to isolate a cDNA encoding the putative murine Golgi CMP-sialic acid transporter. The cloned cDNA encodes a highly hydrophobic, multiple membrane spanning protein of 36.4 kDa, with structural similarity to the recently cloned ammonium transporters. Transfection of a hemagglutinin-tagged fusion protein into the mutant 6B2 led to Golgi localization of the hemagglutinin epitope. Our results, together with the observation that the cloned gene shares structural similarities to other recently cloned transporter proteins, strongly suggest that the isolated cDNA encodes the CMP-sialic acid transporter.**

Biosynthesis of complex asparagine-linked oligosaccharides involves the consecutive activity of several glycosidases which trim the high-mannose structures in the endoplasmic reticulum and Golgi apparatus. Also implicated are numerous Golgi localized glycosyltransferases that catalyze the transfer of monosaccharides from high-energy donors, the nucleotide sugars, to the nascent carbohydrate chains (1, 2). Many glycosyltransferases and glycosidases have been characterized at the molecular level (3, 4). In contrast, very little is known about a third group of proteins essential to the process of glycosylation: the nucleotide sugar transporters.

While nucleotide sugars are synthesized in the cytosol or, in the case of CMP-*N*-acetylneuraminic acid, in the nucleus (5), glycosylation reactions occur in the lumen of the endoplasmic reticulum and Golgi apparatus (6, 7). Therefore translocation of nucleotide sugars across the membranes of the Golgi apparatus is a prerequisite for all glycosylation reactions in this compartment. The existence of specific nucleotide sugar and nucleotide sulfate transport systems has been clearly demonstrated in isolated Golgi vesicles (8, 9) and in permeabilized cells (10). These transport activities can be reconstituted after incorporation of Golgi membrane proteins into liposomes (11, 12), and biochemical analysis revealed a specific transporter for each nucleotide sugar (6). None of the transporter protein has been characterized at the molecular level or purified to homogeneity, with the exception of the adenosine 3′-phosphate 5′-phosphosulfate transporter (13).

The physiological importance of CMP-sialic acid and UDP-galactose transport activities has been shown by the analysis of Chinese hamster ovary (CHO) glycosylation mutants. The complementation groups Lec2 and Lec8 were isolated for their resistance to wheat germ agglutinin (14, 15). The strong reduction in sialylation (Lec2) or galactosylation (Lec8) of glycoproteins and glycolipids observed in these mutants could be attributed to specific defects in the corresponding nucleotide sugar transporters (16, 17). The CMP-sialic acid translocation rate in isolated Golgi vesicles from Lec2 cells was reduced by 98%, while translocation of other nucleotide sugars and of nucleotide sulfate was unaffected (16). These CHO mutants provide the means to isolate the genes of nucleotide sugar transporters by complementation cloning. A previous attempt to clone the CMP-sialic acid transporter by expression cloning in Lec2 cells using toxic lectin selection failed (18). By using an alternative cloning strategy based on the immunoselection procedure introduced by Seed and Aruffo (19), we have successfully isolated a complementary DNA (cDNA) of the gene complementing the defect in Lec2 cells. This strategy has been recently developed in our laboratory to isolate a cDNA encoding polysialyltransferase 1 (20). The data presented in this report present information on the molecular structure of a nucleotide sugar transporter and therefore provide a new basis to characterize this important protein family.

EXPERIMENTAL PROCEDURES

Cell Culture and Complementation Analysis. CHO-K1 and 6B2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) Nut Mix F-12 supplemented with 1 mM sodium pyruvate and 5% fetal calf serum (FCS). Lec2 (ATCC CRL 1736) and Lec8 cells (ATCC CRL 1737) (14) were grown in alpha MEM (GIBCO/BRL) supplemented with 10% FCS.

For complementation analysis, 1:1 mixtures of the two cell lines to be analyzed were seeded in 35-mm culture dishes (total cell number was 10^6) and grown for 16 h. Fusion was induced by incubation with 50% polyethylene glycol in 50 mM Hepes (pH 8.0) (Boehringer Mannheim) as described by Davidson and Gerald (21). Twenty-four hours after fusion, cells were fixed in 50% methanol/50% acetone and stained by sequential incubation with monoclonal antibody (mAb) 735 (22) and anti-mouse alkaline phosphatase (Boehringer Mannheim). Nitroblue tetrazolium (Fluka) and 5-bromo-4-chloro-3-indolylphosphate toluidinium (Fluka) in AP buffer (100 mM Tris·HCl, pH 7.5/100 mM NaCl/5 mM $MgCl_2$) were used as substrates.

Expression Cloning. An oligo(dT)-primed cDNA library from murine AtT20 cells was constructed in eukaryotic expression vector pABE by using a cDNA synthesis kit from Invitrogen. The vector pABE is a derivative of pCDM8 that carries a β-lactamase gene instead of the Sup F fragment. Eight portions of 4×10^6 6B2 cells were cotransfected with 2.5 μg of the cDNA library and 2.5 μg pPSVE1-PyE, encoding the

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Abbreviations: HA, hemagglutinin; PSA, polysialic acid; CMP-SA-Tr; CMP-sialic acid transporter; FITC, fluorescein isothiocyanate.

Data deposition: The sequence reported in this paper has been deposited in the GenBank data base (accession no. Z71268).

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polyoma large T antigen (23), by electroporation at 960 μF and 250 V. Two days later, cells were panned (19) on Petri dishes coated with mAb 735 (22). Plasmids of adherent cells were rescued by the Hirt procedure (24) and amplified in *Escherichia coli* DH5α. Two further rounds of transfection and Hirt extraction were performed. Thereafter, the plasmid pME8, which complements the defect in 6B2 cells, was isolated by sibling selection. DNA sequencing on both strands was performed by the dideoxy chain termination method (25) by using T7 DNA polymerase (Pharmacia) and [α-^{35}S]dATP. Reactions were primed with vector-specific oligonucleotides and subsequently with primers derived from the known sequence of the cDNA.

Western Blot Analysis. SDS/PAGE was performed according to Laemmli (26) on 7% polyacrylamide gels. Proteins were transferred to nitrocellulose membranes by semi-dry blotting (27), and the membranes were blocked by incubation in 2% nonfat dry milk in phosphate-buffered saline (PBS). Immunostaining with mAb 735 was performed as described (28). Sialic acid linked α2,3 to galactose was detected by sequential incubation with digoxigenin-labelled lectin MAA (*Maackia amurensis*) and anti-digoxigenin–F_{ab} alkaline phosphatase-conjugate (Boehringer Mannheim). Bound alkaline phosphatase was revealed by incubation in AP buffer containing nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate toluidinium.

Northern Blot Analysis. Total RNA was isolated from murine tissues and cell lines by guanidinium isothiocyanate extraction and centrifugation through CsCl gradients as described by Chirgwin *et al.* (29). Samples were fractionated on 0.5 M formaldehyde agarose gels and transferred to nylon membranes (30). Blots were hybridized with a digoxigenin-labeled antisense RNA probe (31) transcribed from the entire CMP-sialic acid transporter (CMP-SA-Tr) cDNA subcloned into pBluescript. Hybridization was performed for 16 h at 65°C in 50% formamide, 5× standard saline citrate (SSC), 50 mM sodium phosphate, 7% SDS, 0.1% *N*-lauroylsarcosine, and 2% blocking reagent (Boehringer Mannheim). Membranes were washed in 0.1× SSC and 0.1% SDS at 65°C for 30 min. Bound RNA probes were revealed by incubation with sheep anti-digoxigenin–F_{ab} alkaline phosphatase-conjugate (Boehringer Mannheim) followed by chemiluminescence detection using disodium 3-(4-methoxyspiro {1,2-dioxetone-3,2′-(5′-chloro)-tri-cyclo[3.1.1.1^{3,7}]decan}-4-yl)phenyl phosphate (CSPD) (Boehringer Mannheim).

Expression of a CMP-SA-Tr Fusion Protein with Hemagglutinin Tag. CMP-SA-Tr with a C-terminal influenza hemagglutinin (HA) epitope (YPYDVPDYASL) was prepared by PCR (32) by using the primers ME41 (5′-GCGGATCCATGGCTCCGGCGAGAG-3′) and ME42 (5′-GCGGATCCCACACCAATGATTCTCTCTTTT-3′). The PCR product was digested with *Bam*HI and ligated into the *Bam*HI site of eukaryotic expression vector pEVRF0-HA, which directs the expression of HA-tagged fusion proteins under control of a cytomegalovirus promoter (a kind gift of R. Janknecht, Medizinische Hochschule, Hannover, Germany), resulting in the plasmid pEVRF0-HA-ME8. 6B2 cells were cotransfected with pEVRF0-HA-ME8 and plasmid pAG60, conferring neomycin resistance, using lipofectamine (GIBCO/BRL), following the manufacturer's instructions. Stable transfectants were selected in medium containing 700 μg of G418 per ml.

For immunofluorescence, cells were cultured on glass coverslips, fixed with cold methanol for 1 min, and then incubated in 10% FCS/PBS for 15 min. Cells were incubated with primary antibodies in 0.5% bovine serum albumin (BSA)/PBS and 0.5% saponin for 2 h, washed twice in 0.1% BSA/PBS, and then incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse Ig or rhodamine goat anti-rabbit Ig (Dianova, Hamburg, Germany). Polysialic acid (PSA) was immunolocalized with mAb 735 (22), the HA epitope with mAb 12CA5 (Boehringer Mannheim), and mannosidase II with a rabbit polyclonal antibody (33), kindly provided by K. Moremen (University of Georgia, Athens). Fluorescence labeling was visualized by using a Zeiss Axiophot Epifluorescence microscope with barrier filters to prevent crossover of FITC and rhodamine signals.

RESULTS

Characterization of CHO Mutants. Wild-type CHO cells express PSA on their cell surface (20, 34), which can be detected by mAb 735 (22). To isolate PSA negative CHO mutants, CHO-K1 wild-type cells were treated with ethylmethane sulfonate and PSA positive cells were depleted by panning on mAb 735 (20). PSA negative mutants were cloned and further characterized by lectin analysis. Four independent PSA negative mutants were found that showed a general defect in cell surface expression of sialic acid (clones 1E3, 6B2, 8G8, and 9D3). Fusion experiments indicated that all belong to a single complementation group. To determine whether these mutants belong to a known complementation group, fusion experiments were performed between mutant 6B2 and the CHO glycosylation mutants Lec2 and Lec8 (14). The 1:1 mixtures of the relevant cells were seeded into cell culture dishes, and cell fusion was induced by polyethylene glycol. Two days later, cells were stained with mAb 735 to detect PSA. While each mutant clone (6B2, Lec2, and Lec8) is negative for PSA, reexpression of PSA would indicate complementation to the wild type in cell hybrids. Fusion of 6B2 cells with Lec8 cells rescued PSA expression, while a 6B2×Lec2 fusion did not (data not shown). This clearly indicates that 6B2 and Lec2 cells belong to the same complementation group.

Cloning of CMP-SA-Tr. To identify the activity able to complement 6B2/Lec2 cells to the wild-type phenotype, a cDNA library of the murine cell line AtT20 was constructed in the expression vector pABE, which is a derivative of pCDM8. This cDNA library was transfected into 6B2 cells together with the polyoma large T antigen expressing plasmid pPSVE1-PyE (23) to achieve amplification of the cDNA plasmids. Transfectants reconstituted to the wild-type phenotype were enriched by panning on mAb 735 (see *Experimental Procedures*). After three rounds of panning, a single cDNA clone, named pME8, was isolated by sibling selection. Transient transfection of pME8 into 6B2 and Lec2 cells resulted in reexpression of PSA and reconstituted terminal α-2,3-linked sialic acid expression (Fig. 1), indicating full complementation to the wild-type phenotype. We have named this cDNA CMP-SA-Tr.

Analysis of the CMP-SA-Tr cDNA. The nucleotide and predicted amino acid sequence of the pME8 insert are shown in Fig. 2*A*. The 1185-bp cDNA insert revealed a single open

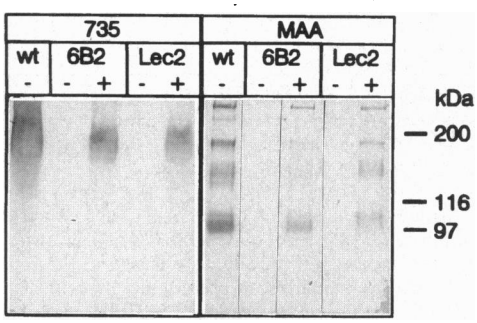

FIG. 1. Complementation of 6B2 and Lec2 cells by transient transfection with plasmid pME8. 6B2 and Lec2 cells were transfected with pME8 (+) or with the empty vector pABE (−). Two days after transfection, cell lysates were resolved on SDS/PAGE and transferred to nitrocellulose. Membranes were stained with the PSA-specific antibody 735 or digoxigenin-labeled lectin from *M. amurensis* (MAA), which specifically recognizes sialic acid α2,3-linked to galactose (35).

A

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   1 ATGGCTCCGGCGAGAGAAAATGTCAGTTTATTCTTCAAGCTGTACTGCTTGGCGGTGATG
   1 MetAlaProAlaArgGluAsnValSerLeuPhePheLysLeuTyrCysLeuAlaValMet
                *

  61 ACTCTGGTGGCTGCCGCTTACACCGTAGCTTTAAGATACACAAGGACAACAGCTGAAGAA
  21 ThrLeuValAlaAlaAlaTyrThrValAlaLeuArgTyrThrArgThrThrAlaGluGlu

 121 CTCTACTTCTCAACCACTGCCGTGTGTATCACAGAAGTGATAAAGTTACTGATAAGTGTT
  41 LeuTyrPheSerThrThrAlaValCysIleThrGluValIleLysLeuLeuIleSerVal

 181 GGCCTGTTAGCTAAGGAAACTGGCAGTTTGGGTAGATTTAAAGCCTCATTAAGTGAAAAT
  61 GlyLeuLeuAlaLysGluThrGlySerLeuGlyArgPheLysAlaSerLeuSerGluAsn

 241 GTCTTGGGGAGCCCCAAGGAACTGGCGAAGTTGAGTGTGCCATCACTAGTGTATGCTGTG
  81 ValLeuGlySerProLysGluLeuAlaLysLeuSerValProSerLeuValTyrAlaVal

 301 CAGAACAACATGGCCTTCCTGGCTCTCAGTAATCTGGATGCAGCAGTGTACCAGGTGACC
 101 GlnAsnAsnMetAlaPheLeuAlaLeuSerAsnLeuAspAlaAlaValTyrGlnValThr

 361 TATCAACTGAAGATCCCCTGCACTGCTTTATGTACTGTTTTAATGTTAAATCGAACACTC
 121 TyrGlnLeuLysIleProCysThrAlaLeuCysThrValLeuMetLeuAsnArgThrLeu
                                                  *

 421 AGCAAATTACAGTGGATTTCCGTCTTCATGCTGTGTGGTGGGGTCACACTCGTACAGTGG
 141 SerLysLeuGlnTrpIleSerValPheMetLeuCysGlyGlyValThrLeuValGlnTrp

 481 AAACCAGCCCAAGCTTCAAAAGTCGTGGTAGCGCAGAATCCATTGTTAGGCTTTGGTGCT
 161 LysProAlaGlnAlaSerLysValValValAlaGlnAsnProLeuLeuGlyPheGlyAla

 541 ATAGCTATTGCTGTATTGTGCTCTGGATTTGCAGGAGTTTATTTTGAAAAAGTCTTAAAG
 181 IleAlaIleAlaValLeuCysSerGlyPheAlaGlyValTyrPheGluLysValLeuLys

 601 AGTTCCGACACTTCCCTTTGGGTGAGAAACATTCAGATGTATCTGTCAGGGATCGTTGTG
 201 SerSerAspThrSerLeuTrpValArgAsnIleGlnMetTyrLeuSerGlyIleValVal

 661 ACGTTAGCTGGTACCTACTTGTCAGATGGAGCTGAAATTCAAGAAAAAGGATTCTTCTAT
 221 ThrLeuAlaGlyThrTyrLeuSerAspGlyAlaGluIleGlnGluLysGlyPhePheTyr

 721 GGCTACACGTATTATGTCTGGTTTGTTATCTTCCTTGCTAGTGTGGGAGGCCTCTACACG
 241 GlyTyrThrTyrTyrValTrpPheValIlePheLeuAlaSerValGlyGlyLeuTyrThr

 781 TCAGTGGTGGTGAAGTATACAGACAACATCATGAAAGGCTTCTCTGCTGCCGCAGCCATT
 261 SerValValValLysTyrThrAspAsnIleMetLysGlyPheSerAlaAlaAlaAlaIle

 841 GTTCTTTCTACCATTGCTTCAGTCCTACTGTTTGGATTACAGATAACACTTTCATTTGCA
 281 ValLeuSerThrIleAlaSerValLeuLeuPheGlyLeuGlnIleThrLeuSerPheAla

 901 CTGGGAGCTCTTCTTGTGTGTGTTTCCATATATCTCTATGGGTTACCCAGACAAGATACT
 301 LeuGlyAlaLeuLeuValCysValSerIleTyrLeuTyrGlyLeuProArgGlnAspThr

 961 ACATCCATTCAACAAGAAGCAACTTCAAAAGAGAGAATCATTGGTGTGTGATTTGAATCT
 321 ThrSerIleGlnGlnGluAlaThrSerLysGluArgIleIleGlyVal

1021 CAAGAGATTCCTATAAGGACTTAAACTGTTGATAATAAATTAGAGCCTTAAGTCAACCCC

1081 GATGGTAGGTTAAATAATGTCAACAAAATAATTGTATGACATAAGAATCAAGAAGAAAAC

1141 TCTGAATGAAATGCTAAAACAGATTTAAAAAAAAAAAAAAAAA
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B

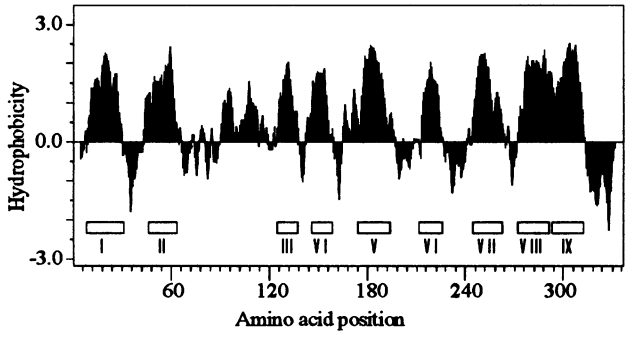

FIG. 2. Sequence analysis of CMP-SA-Tr. (*A*) Nucleotide and deduced amino acid sequence of CMP-SA-Tr. The two potential *N*-glycosylation sites are indicated by asterisks, and the four leucine residues of the leucine zipper motif are double underlined. Potential polyadenylylation signals are underlined. The EMBL data base accession number of the nucleotide sequence is Z71268. (*B*) Hydropathy plot of CMP-SA-Tr. The plot was calculated with a window size of nine amino acids using the hydrophobicity values of Kyte and Doolittle (36). Boxes indicate the positions of putative membrane spanning segments predicted according to Klein *et al.* (37).

reading frame of 1011 bp with an ATG at position 1. Although we have extended the 5′-sequence by using 5′-rapid amplification of cDNA ends (RACE) (38), we have not found additional in frame ATG codons, but the sequence around the predicted start codon showed to be in good correlation with the translation start consensus motif (39) (data not shown). The 3′-noncoding region of the cDNA contains a single AATAAA sequence and two AATAAT streches, observed in 2% of mRNAs (40). However, the motifs are more than 50 bases upstream from the poly(A) tail. It is unclear whether one of these sequence motifs functions as polyadenylylation signal or whether the 3′ end of the cDNA is incomplete due to priming by an A-rich region.

The deduced amino acid sequence of CMP-SA-Tr encodes a highly hydrophobic 36.4-kDa protein (Fig. 2*A*). An N-terminal hydrophobic region that could serve as a signal sequence has not been found. Structural analysis according to Klein *et al.* (37) predicted six to nine transmembrane domains (Fig. 2*B*). Segments III, IV, and VI are only "possibly integral" (37, 41), since the hydrophobic stretches contain less than 17 residues. In this context it must be noted that Golgi resident proteins have shorter transmembrane domains than proteins of the plasma membrane (42), and that this structural property seems to be involved in Golgi retention (43, 44). Although the protein has two potential asparagine-linked glycosylation sites, preliminary results provide no evidence for *N*-glycosylation of CMP-SA-Tr (M.E. and R.G.-S., unpublished results).

Interestingly, the CMP-SA-Tr protein contains a leucine zipper motif, which is part of the second potential transmembrane helix and of the following hydrophilic stretch (Fig. 2*A*). This structural feature has recently been found to be part of integral membrane proteins, for example the erythrocyte-type vertebrate glucose transporters (45) and the ammonium transporter MEP1 of *Saccharomyces cerevisiae* (46). In every instance, this motif is located within or around the second transmembrane domain of the transport proteins. Furthermore, although no significant sequence homology exists among CMP-SA-Tr, MEP1, and the recently cloned ammonium transporter from *Arabidopsis thaliana* (47), hydrophobicity plots of the N-terminal 300 amino acids look very similar. There is striking similarity in the proposed membrane topology, in particular a relatively long hydrophilic stretch between transmembrane domains II and III and the proximity of domains VIII and IX. Finally, another putative nucleotide sugar transporter, the UDP-GlcNAc transporter of the yeast *Kluyveromyces lactis*, has been reported to contain a leucine zipper motif (48).

The CMP-SA-Tr sequence was entered into both the EMBL and SwissProt data bases. The only significant homology was a 40.7% identity to a *Caenorhabditis elegans* open reading frame on chromosome III that encodes a 29.4-kDa protein [accession nos. Q02334 (SwissProt) and M98552 (EMBL); reference 49].

Expression of CMP-SA-Tr. The expression of CMP-SA-Tr mRNA was analyzed by Northern blotting in different murine tissues (Fig. 3*A*), CHO wild-type cells, and mutants 6B2 and Lec2 (Fig. 3*B*). A prominent band of 2.3 kb and, with the exception of CHO cells, a minor band of about 1.4 kb appeared in all tissues and cell lines examined. Ethidium bromide staining indicated equal loading of the gels (data not shown). Presence of the hybridization signal in CHO mutants strongly suggests that the mutant phenotypes are due to point mutations within the coding region of the gene.

To determine the subcellular localization of CMP-SA-Tr, a CMP-SA-Tr fusion protein was expressed with a C-terminal HA tag. Stable transfection of this construct into 6B2 cells led to cell surface expression of PSA, indicating that the modified protein was functionally active (Fig. 4*A*). When stained with the anti-HA antibody 12CA5, these transfectants showed an immunofluorescence pattern characteristic of the Golgi apparatus (Fig. 4*C*), which colocalize with that of mannosidase II

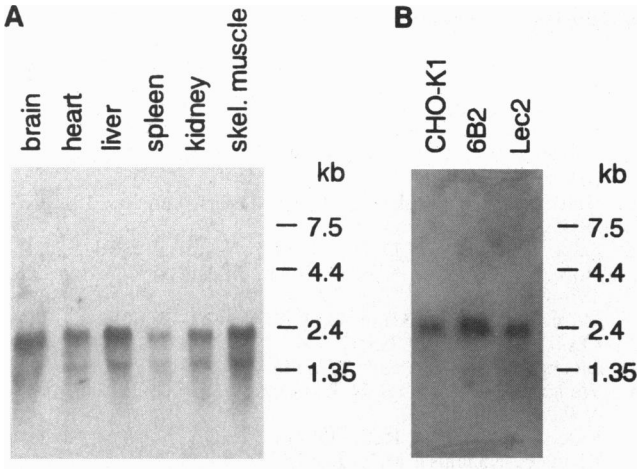

FIG. 3. Northern blot analysis of total RNA (10 μg/lane) from different murine tissues (*A*), CHO wild-type cells, and 6B2 and Lec2 mutants (*B*). Samples were resolved on agarose formaldehyde gels and transferred to nylon membranes. Membranes were hybridized with a digoxigenin-labeled antisense RNA probe, transcribed from the CMP-SA-Tr cDNA.

(Fig. 4*D*). This result is consistent with the Golgi association of CMP-sialic acid transport activity (6).

DISCUSSION

CHO glycosylation mutants belonging to the Lec2 complementation group exhibit a strong reduction in sialylation of glycoproteins and glycolipids (15, 50). The phenotype of these mutants results from the inability of Golgi vesicles to translocate CMP-sialic acid across the Golgi membrane (16). By expression cloning we identified a cDNA that complements the Lec2 phenotype. The following points strongly suggest that the cloned cDNA is the CMP-sialic acid transporter. (*i*) The deficiency in CMP-sialic acid transport in Lec2 mutants was found to be specific because transport of other nucleotide sugars was unaffected (16). (*ii*) The CMP-SA-Tr cDNA encodes a highly hydrophobic, multiple membrane spanning protein, and the overall membrane topology shows a striking similarity to two recently cloned yeast and plant ammonium transporters (46, 47). (*iii*) Expression of the cloned cDNA as a fusion protein with a HA epitope revealed the Golgi localization expected for the CMP-sialic acid transporter. Nevertheless, the final demonstration that the cloned cDNA encodes the CMP-sialic acid transporter requires functional reconstitution of the purified recombinant protein into liposomes.

Because of structural similarities, the CMP-SA-Tr can be grouped into the superfamily of transporter proteins that are responsible for the translocation of various organic and inorganic compounds, like ammonium and glucose (46). Many of these transporter proteins contain a leucine zipper motif that is known to play an important role in dimerization of transcription factors (51). Leucine zippers have only recently been identified in integral membrane proteins known to form oligomers (45, 52). Support for the functional relevance of the CMP-SA-Tr leucine zipper motif comes from the recent observation that this structure is also present in the yeast UDP-GlcNAc transporter (48). The hypothesis that CMP-SA-Tr may dimerize is attractive because the functional units of other Golgi resident enzymes, including the nucleotide sulfate (adenosine 3′-phosphate 5′-phosphosulfate) transporter, appear to be homodimeric (13, 53).

Due to their exposed position and physicochemical properties, sialic acids play important roles in cellular interactions and adhesion processes. For example, sialic acids are major determinants of selectin ligands and essential components of viral receptors (54, 55). The amount of sialic acid expressed on the cell surface depends on the cellular differentiation status, and it has been shown that the malignant potential of tumor cells directly correlates with the level of cell surface sialylation (56, 57). Most studies on the regulation of sialylation concentrate on the analysis of sialyltransferases. However, sialylation could also be regulated at the level of substrate availability. The CMP-SA-Tr provides an excellent target to control overall cell surface expression of sialic acid by regulating the expression of the transporter or through the action of specific inhibitors. Consistent with this, inhibitors of CMP-sialic acid translocation were shown to decrease metastases (58, 59). The molecular characterization of this transporter provides a new opportunity to selectively block its expression or activity.

This report on CMP-SA-Tr presents the molecular characterization of a nucleotide sugar transporter in higher eukaryotes. Only one other nucleotide sugar transporter, the UDP-GlcNAc transporter of *Kluyveromyces lactis* (48), has been cloned to date. Regions of sequence conservation between these two proteins should facilitate the identification of other nucleotide sugar transporters and related proteins. The cloning of CMP-SA-Tr provides another example of the efficiency of our complementation cloning procedure, which is based on the expression of PSA attached to the neural cell adhesion molecule (NCAM) in wild-type CHO cells (20, 34). Many glycosylation mutants derived from CHO cells and murine cell lines have been described (60, 61). Since polysialylation of NCAM is the final step in the maturation of this glycoprotein, most defects in genes involved in the glycosylation pathway lead to the disappearance of PSA from the cell

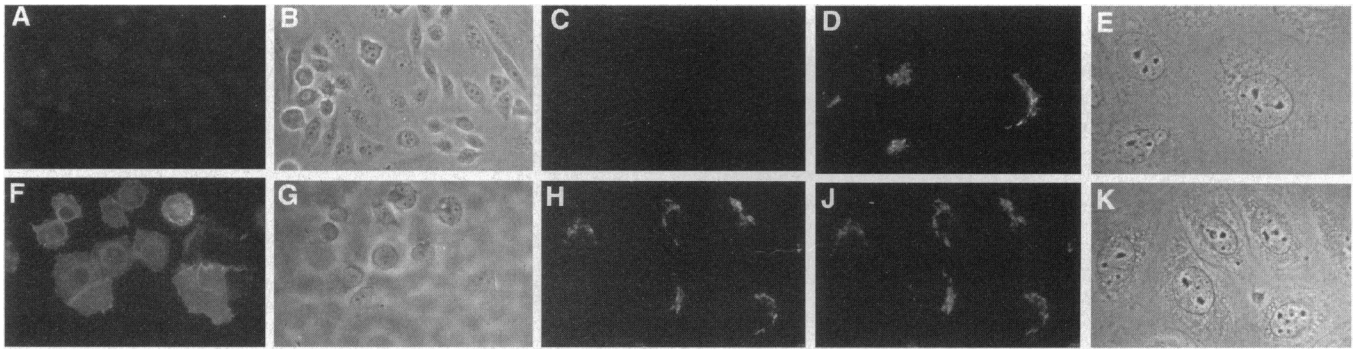

FIG. 4. Subcellular localization of CMP-SA-Tr. 6B2 cells were stably transfected with pEVRF0-HA-ME8, encoding CMP-SA-Tr with a C-terminal HA tag (*F–K*) or empty vector pEVRF0-HA (*A–E*). Cells were stained with mAb 735 and anti-mouse Ig-FITC to visualize PSA expression (*A* and *B*). Double-labeling of HA tag and mannosidase II was performed with the anti-HA mAb 12CA5 and an antiserum directed against Golgi mannosidase II followed by anti-mouse Ig-FITC (visualized in *C* and *H*) and anti-rabbit Ig-rhodamine (visualized in *D* and *J*). (*A*, *B*, *F*, and *G*, ×160; *C–E* and *H–K*, ×400.)

surface. Our methodology could potentially be used to clone any of these genes, provided the relevant mutants are available. Candidate genes include other nucleotide sugar transporters, glycosyltransferases and nucleotide sugar synthetases.

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