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Molecular cloning of the Golgi apparatus uridine diphosphate-*N*-acetylglucosamine transporter from *Kluyveromyces lactis*

(glycosylation/glycoproteins/glycolipids/yeast)

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ABSTRACT **The mannan chains of *Klyveromyces lactis* mannoproteins are similar to those of *Saccharomyces cerevisiae* except that they lack mannose phosphate and have terminal α1→2-linked *N*-acetylglucosamine. The biosynthesis of these chains probably occurs in the lumen of the Golgi apparatus, by analogy to *S. cerevisiae*. The sugar donors, GDP-mannose and UDP-GlcNAc, must first be transported from the cytosol, their site of synthesis, via specific Golgi membrane transporters into the lumen where they are substrates in the biosynthesis of these mannoproteins. A mutant of *K. lactis*, mnn2-2, that lacks terminal *N*-acetylglucosamine in its mannan chains *in vivo*, has recently been characterized and shown to have a specific defect in transport of UDP-GlcNAc into the lumen of Golgi vesicles *in vitro*. We have now cloned the gene encoding the *K. lactis* Golgi membrane UDP-GlcNAc transporter by complementation of the mnn2-2 mutation. The mnn2-2 mutant was transformed with a genomic library from wild-type *K. lactis* in a pKD1-derived vector; transformants were isolated and phenotypic correction was monitored following cell surface labeling with fluorescein isothiocyanate conjugated to *Griffonia simplicifolia* II lectin, which binds terminal *N*-acetylglucosamine, and a fluorescent activated cell sorter. A 2.4-kb DNA fragment was found to restore the wild-type lectin binding phenotype. Upon loss of the plasmid containing this fragment, reversion to the mutant phenotype occurred. The above fragment contained an open reading frame for a multitransmembrane spanning protein of 328 amino acids. The protein contains a leucine zipper motif and has high homology to predicted proteins from *S. cerevisiae* and *C. elegans*. In an assay *in vitro*, Golgi vesicles isolated from the transformant had regained their ability to transport UDP-GlcNAc. Taken together, the above results strongly suggest that the cloned gene encodes the Golgi UDP-GlcNAc transporter of *K. lactis*.**

Outer mannan chains of mannoproteins of *Kluyveromyces lactis* are similar to those of *Saccharomyces cerevisiae*, with the exception that they have a terminal α1→2-linked *N*-acetylglucosamine residue and lack mannose phosphate groups (1). By analogy to *S. cerevisiae*, it is probable that the outer mannan chains and terminal *N*-acetylglucosamine residues are added in the lumen of Golgi apparatus (2). GDP-mannose and UDP-GlcNAc, the sugar donors in these reactions, must first be transported from the cytosol, their sites of synthesis, via Golgi membrane transporters into the lumen to serve as substrates for the biosynthesis of the mannan chains (3). Mutants of *K. lactis* without terminal *N*-acetylglucosamine in their mannoproteins have been previously isolated and described by Ballou and coworkers (4, 5). In one of these mutants, mnn2-2, the biosynthesis of glucosamine containing lipids is also impaired (6). This mutant was recently found to have a defect in transport of UDP-GlcNAc into Golgi vesicles; the transport defect appears to be specific since the same vesicles transport GDP-mannose into their lumen at initial velocities comparable to those of wild-type vesicles (6).

We hypothesized that the difference in the phenotype between the above described mutant and the corresponding wild-type cells would result in differential binding to *Griffonia simplicifolia* II (GS II) lectin, which recognizes terminal α- or β-linked *N*-acetylglucosamine. Indeed, mutant and wild-type cells can be separated in a fluorescent activated cell sorter (FACS) following labeling with GS II-fluorescein isothiocyanate (FITC) lectin. The *K. lactis* mnn2-2 cells were transformed with a genomic library from wild-type *K. lactis*; transformants were isolated and phenotypic correction was monitored by using a FACS following incubation of cells with GS II-FITC lectin. A 2.4-kb DNA fragment was isolated, sequenced, and found to encode a multitransmembrane spanning 328-amino acid protein. Upon loss of the plasmid containing this fragment from the transformant, reversion to the mutant phenotype occurred. In an assay *in vitro*, we determined that Golgi vesicles isolated from the transformant had regained their ability to transport UDP-GlcNAc into their lumen. Together, the above results strongly suggest that the multimembrane spanning protein encodes the Golgi UDP-GlcNAc transporter of *K. lactis*.

MATERIALS AND METHODS

Radioactive Substrates. UDP-[6-^{3}H]-*N*-acetylglucosamine (29 Ci/mmol; 1 Ci = 37 GBq), GDP-[2-^{3}H]mannose (19 Ci/mmol), and [^{3}H]sodium acetate (135 Ci/mol) were purchased from DuPont/New England Nuclear.

Strains Utilized, Production of Spores, and Tetrad Dissections. The parental strains of *K. lactis* utilized in this study were as follows: Y58, (54) *MAT***a**, *mnn2-2*, *his 4C* (4) obtained from C. Ballou (University of California, Berkeley) and MG1/2, *MATα*, *uraA*, *arg*$^-$, *lys*$^-$ K$^+$ pKD1$^+$ (7) obtained from C. Falcone (University of Rome I).

These strains were mass mated as described (8) on malt extract (ME) agar to obtain the *K. lactis* strain KL_3 (*MATα* *uraA*, *mnn2-2*, *arg*$^-$ K$^+$ pKD1$^+$) used throughout this study. ME was used as mating and sporulation medium. Spore isolation involved digestion of the sporulated culture with zymolase 100 T at 0.1 mg/ml for 10 min at room temperature followed by dissection of tetrads with a micromanipulator.

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Abbreviations: FITC, fluorescein isothiocyanate; GS II, *Griffonia simplicifolia* II; FACS, fluorescence activated cell sorter; ORF, open reading frame.

Data deposition: The sequence reported in this paper has been deposited in the GenBank data base (accession number U48413).

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Culture Media and DNA Manipulations. Yeast strains were grown in yeast extract/peptone/glucose or SD medium (0.67% yeast nitrogen base without amino acids and 2% glucose) supplemented with the required amino acids (9). *Escherichia coli* DH 5 α (BRL) was used in the cloning experiments. Standard molecular cloning techniques were used (10).

Construction of Genomic Library. A genomic library was constructed from *K. lactis* strain MG1/2 in KEp 6 vector (11). The KEp 6 vector has a segment of pKD1 that carries the replication origin of the 1.6 μm plasmid (7). Stable replication of this vector requires the presence of resident pKD1 in the recipient yeast cell. The copy number and stability of this plasmid is comparable to the 2 μm plasmid derived vectors of *S. cerevisiae*. KEp 6 also has the URA 3 gene of *S. cerevisiae* that complements the uraA mutation of *K. lactis*; it also contains pBR 322 sequences to allow selection and amplification in *E. coli*.

A partial *Sau*3A digest of genomic DNA of *K. lactis* MG1/2 (average size, 7 kb) was cloned into the *Bam*HI site of the KEp 6 vector. Ten pools of ≈2200 transformants each were made and ≈80% of the clones had inserts based on the ratio of ampillicin to tetracyclene resistance. Plasmid DNA was prepared from each of the 10 pools and used to transform by electroporation strain KL 3 of *K. lactis*.

Cell Surface Labeling of *K. lactis* with GS II-FITC. Cells were grown in yeast extract/peptone/extract medium and then washed twice with 0.9% NaCl containing 2 mM $CaCl_2$. Approximately 5 OD_{600} of cells was resuspended in 100 μl of 0.5 mg/ml GS II-FITC (EY Laboratories) in 0.9% NaCl containing 2 mM $CaCl_2$ and incubated for 1 h at 30°C with shaking. Samples were then washed twice and resuspended in 5 ml of 0.9% NaCl containing 2 mM CaCl. The florescent emission was determined by an RF-540 spectrofluorophotometer (Shimadzu) or by FACS analysis.

Fluorescent Activated Cell Sorting. Cells were run through either a FACStar Plus or a FACS Vantage (Becton Dickinson) equipped with an Innova 90 argon laser (Coherent, Santa Clara, CA) tuned to 488 nm with 100 mw output power at the Massachusetts Institute of Technology Flowcytometry Facility. Emission of FITC was measured using a 560 nm short pass dichroic and a 530 ± 30 nm DF filter (Omega Optical, Brattleboro, VT). Cells were passed through the flow cytometers at a rate of 3000–4000/sec with a 2-droplet sort envelope. The sort region was based on the top 40% of the highest expressing cells. When individual transformants were studied, 20,000 event data files were collected and analyzed with Lysis II on a Hewlett–Packard 340 computer.

Transformation of *K. lactis* by Electroporation. Electrocompetent *K. lactis* cells were prepared from 10 ml of an overnight stationary phase culture by washing the cells two times with ice cold sterilized water and two times with ice cold 10% glycerol; cells were resuspended in 0.5 ml of 10% glycerol. Electrocompetent cells (40 μl) were mixed with ≈1 μg of plasmid DNA and placed on a 0.2-cm gap electroporation cuvette. A pulse of 2.5 KV, 25 μF, 200 ohms was delivered with a Gene Pulser (Bio-Rad). The time constant was ≈5 msec.

DNA Sequencing. A 2.4-kb *Pst*I–*Hin*dIII fragment of the genomic clone pCA 66 was subcloned into pBluescript; DNA sequencing of both strands was done with dye primers using the dideoxy chain termination method (12). An Applied Biosystems DNA sequencer was used at the Molecular Genetics Facility at the University of Georgia, Athens.

DNA sequence results were assembled into a contiguous sequence data base with SEQMAN program (DNAstar, Madison, WI). Sequence comparisons against the GenBank sequence data bases were performed using the BLAST programs (13). The hydrophobicity plots were generated by the method of Kyte and Doolittle (14) with a window of 17 amino acids.

Subcellular Fractionation and Assays for Nucleotide Sugar Translocation, GDPase, and α1–2-*N*-Acetylglucosaminyl Transferase. All the above procedures have recently been described in detail (6). Briefly, cultures were converted to spheroplasts and a P_3 fraction (15) was obtained and characterized. These fractions were obtained from *K. lactis* strains MG1/2 (wild type), KL_3 (mnn2-2), and KL_3 containing pCA 73 (*URA3*, *MNN2-2*) grown in SD medium. They were equally enriched in Golgi markers such as GDPase (3.2-fold; refs. 16 and 17) and α1–2-*N*-acetylglucosamine transferase (4.1-fold; refs. 4 and 6). Latency of GDPase was at least 95% before nucleotide sugar translocation assays were done, which indicated that the vesicles were sealed and were of the same membrane topographic orientation as *in vivo*.

RESULTS

We recently characterized the biochemical defect of *K. lactis* mnn2-2, a mutant lacking terminal *N*-acetylglucosamine in its mannan chains, as a deficiency of transport of UDP-GlcNAc into the Golgi apparatus (6). In the Golgi lumen, this nucleotide sugar is a substrate for the α1–2-*N*-acetylglucosaminyl transferase that adds terminal *N*-acetylglucosamine to outer mannan chains.

To clone the Golgi membrane UDP-GlcNAc transporter gene by complementation, one needs a *K. lactis* strain that retains the mnn2-2 mutation, is ura^-, and contains the $pKD1^+$ (1.6 μm) plasmid. Such a strain could then sustain replication of a library made in the KEp 6 vector, which only contains the pKD1 origin of replication. This cloning system is analogous to the widely used 2 μm of *S. cerevisiae*. A KL_3 strain was constructed as described in *Methods* and transformed by electroporation with a genomic library made in the Kep 6 vector. Approximately 15,000 ura^+ transformants were pooled and screened for phenotypic correction by taking advantage of the differences in binding at the cell surface of the GS II lectin between wild-type and the mnn2-2 mutant. As can be seen in Fig. 1*A*, wild-type *K. lactis* mannans have one terminal *N*-acetylglucosamine per repetitive mannan subunit. This sugar specifically binds to the above fluorescent lectin and is absent in the mnn2-2 mutant. Chitin, a *N*-acetylglucosamine homopolyer of the cell wall, does not bind to the lectin. Using GS II-FITC lectin to label the cell surface a 12-fold increase in the mean intensity of fluorescence emission per particle as measured by FACS was obtained between mutant and wild-type cell populations (Fig. 1*B*). This window was used to screen for reappearance of wild-type fluorescence of transformants. It was assumed that the correcting plasmid would encode the Golgi UDP-GlcNAc transporter protein.

An aliquot (≈5×10^6 cells) from a pool of 15,000 ura^+ transformants was incubated with GS II-FITC lectin and subjected to FACS analysis. Approximately 2% of the population with fluorescence intensity in the wild-type cell range was collected. These cells were grown for 3 h under selective conditions, incubated with GS II-FITC lectin, and subjected to another FACS cycle. This time, 3×10^6 cells were analyzed and ≈0.05% of the population with fluorescence intensity in the wild-type cell range was collected. Individual clones were analyzed for GS II-FITC lectin labeling. After screening 600 individual clones, 4 were determined to have recovered the wild-type cell surface fluorescent labeling pattern (Fig. 1*C*).

Restriction endonuclease analyses of the recovered plasmids indicated that they were identical and contained an insert of approximately 9.5 kb. This plasmid, which contained the complementing genomic clone, was named pCA 66 and is shown in Fig. 2. pCA 66 was found to be necessary and sufficient for phenotypic correction of the cell surface fluorescence labeling of the mutant. Transformation of KL_3 (mnn2-2, ura^-) with pCA 66 (*MNN2-2*, *URA3*) resulted in cells with wild-type fluorescence intensity labeling with GS II-FITC lectin. This correction was plasmid dependent; when the transformants were grown nonselectively and plated in 5-fluo-

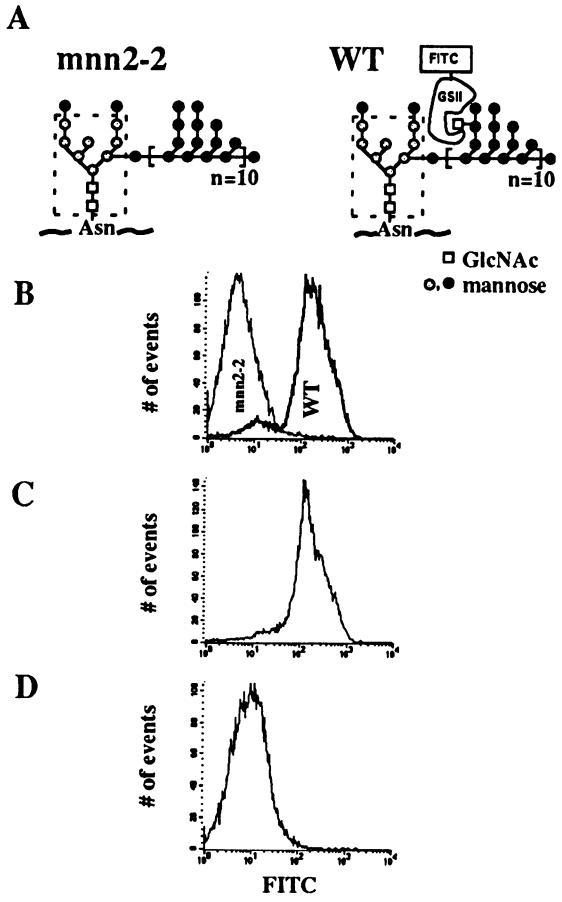

FIG. 1. Cell surface labeling and separation of *K. lactis* cells by FACS. (*A*) Mannan chains of mutant KL_3 (mnn2-2) and wild-type *K. lactis* cells. Terminal *N*-acetylglucosamine on the wild-type chains binds to GS II-FITC lectin. (*B*) Fluorescence emission of mutant KL_3 (mnn2-2) and wild-type MG1/2 strains following GS II-FITC lectin binding and FACS. (*C*) Fluorescence emission of KL_3 cells transformed with pCA 66 (*URA3*, *MNN2-2*). (*D*) Fluorescence emission of transformant of *C* after plating on 5-fluoroorotic acid (loss of plasmid) and labeling with GS II-FITC lectin.

roorotic acid to select for cells that had lost the URA-containing pCA 66 plasmid, mutant levels of cell surface GS II-FITC fluorescence was obtained (Fig. 1*D*).

The genomic clone (Fig. 2) was mapped by subcloning fragments of pCA 66 into KEp 6 and measuring their ability to confer the wild-type phenotype. A 4.3-kb *Hin*dIII/*Bam*HI fragment (pCA 70, Fig. 2) did not correct the phenotype, but a 3.8-kb *Bam*HI/*Hin*dIII fragment (pCA 71, Fig. 2) did. Further subcloning into KEp 6 allowed us to identify a 2.4-kb *Pst*I/*Hin*dIII fragment (pCA 73, Fig. 2) that resulted in phenotypic correction. Initial sequencing of this fragment revealed a single open reading frame (ORF) (Fig. 2) contained entirely within the 1.4-kb *Apa*L1/*Hin*dIII fragment. The complete sequence of this fragment is shown in Fig. 3. The ORF found encodes a highly hydrophobic, 328-amino acid protein that is probably the Golgi UDP-GlcNAc transporter. The protein has two potential *N*-glycosylation sites at amino acids 86 and 254. A leucine zipper motif was found between amino acids 267 and 288.

It was important to determine whether the phenotypic correction, as measured by restored ability to bind GS II-FITC lectin at the cell surface of the KL_3 mutant transformed with pCA 73 was also accompanied by restoration of UDP-GlcNAc transport into Golgi vesicles. For this purpose, Golgi-enriched vesicles of comparable purity were isolated from wild-type mnn2-2 mutant and the mutant transformed with pCA 73. As

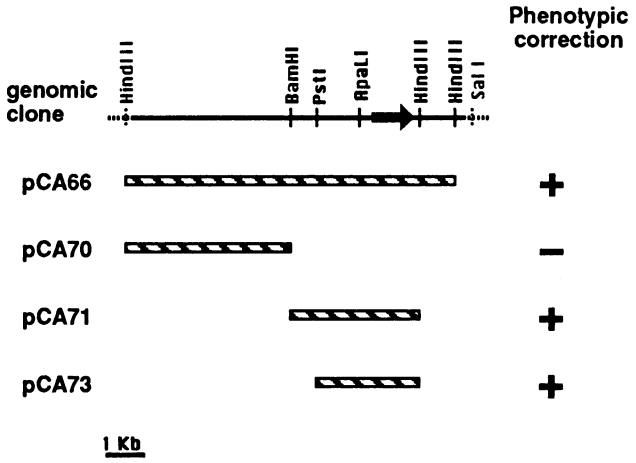

FIG. 2. Genomic clone containing the *MNN2-2* ORF. Restriction sites in boldface type are present in the genomic clone; *Hin*dIII and *Sal*I sites belong to the KEp 6 vector. The hatched bars represent inserts in pCA 66, pCA 70, pCA 71, and pCA 73 that were cloned into the KEp 6 vector. Phenotypic correction was measured by surface labeling with GS II-FITC lectin and FACS. The arrow indicates the position and orientation of the *MNN2-2* ORF determined after sequencing the *Apa*LI/*Hin*dIII fragment.

can be seen in Table 1, Golgi vesicles from the transformant had regained their ability to transport UDP-GlcNAc into their lumen.

DISCUSSION

Recent evidence has suggested that Golgi membrane nucleotide sugar transporters may play an essential role in posttranslational modifications occurring in the Golgi lumen. Experiments *in vivo* (16, 17) and *in vitro* (18) with *S. cerevisiae* have shown that inhibition of the GDP-mannose/GMP antiporter brought about by gene disruption of the Golgi lumen GDPase results in inhibition of mannosylation of both proteins and lipids *in vivo* and drastically reduces transport of GDP-mannose into the Golgi lumen *in vitro*. More recently (19), we have found that mutant MDCK cells, which have only 2% of the wild-type activity of Golgi UDP-galactose transport, have very different galactosylation patterns of macromolecules in the Golgi lumen *in vivo*; this is probably a consequence of limited availability in the Golgi lumen of UDP-galactose and different K_m values of Golgi galactosyltransferases for proteins, lipids, and glycosaminoglycans.

The following lines of evidence strongly suggest that we have cloned the UDP-GlcNAc transporter of the Golgi apparatus membrane from *K. lactis*: a multitransmembrane spanning protein (see below) corrects the phenotype of a mutant *that in vivo* has a deficiency in terminal mannoprotein and lipid *N*-acetylglucosamine residues and *in vitro* has a specific defect in UDP-GlcNAc transport into Golgi-enriched vesicles (5, 6). Phenotypic correction was determined by both the ability of

Table 1. Transport of UDP-GlcNAc into Golgi-enriched vesicles; concentration of radioactive solutes within vesicles after a 3-min incubation

		Solute, pmol/mg protein		
Cell type	Strain	S_τ	S_o	S_i
Wild type	MG 1/2	2.03 ± 0.21	0.9	1.13 ± 0.21
mnn2-2	KL_3	0.91 ± 0.02	0.9	0.01 ± 0.02
Transformant	KL_3 [pCA73]	2.61 ± 0.30	0.9	1.71 ± 0.30

UDP-[^{3}H]GlcNAc (600 dpm/pmol, 0.5 μM) was incubated for 3 min at 30°C with a P_3 vesicle fraction (1.0 mg of protein) as described (3). S_t, S_o, and S_i are, respectively, total solutes in the pellet, solutes outside, and solutes inside the vesicle pellet. Results are an average ± SE of triplicate determinations.

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TGTGCACGTCTTGGCTATTG GATATAAGATTTTCGCGGGT TGATGACATAGAGCGTGTAC TACTGTAATAGTTGTATATT   80

CAAAAGCTGCTGCGTGGAGA CAGACTAAAATAGATAAAAA GCACACATTTTGACTTCGGT ACCGTCAACTTAGTGGGACA  160
      ┌ - - ┐
GTCTTTTATATTTGGTGTAA GCTCATTTCTGGTACTATTC GAAACAGAACAGTGTTTTCT GTATTACCGTCCAATCGTTT  240
      └ - - ┘
GTCATGAGTTTTGTATTGAT TTTGTCGTTAGTGTTCGGAG GATGTTGTTCCAATGTGATT AGTTTCGAGCACATGGTGCA  320
   M  S  F  V  L  I   L  S  L  V  F  G   G  C  C  S  N  V  I   S  F  E  H  M  V  Q   26

AGGCAGCAATATAAATTTGG GAAATATTGTTACATTCACT CAATTCGTGTCTGTGACGCT AATTCAGTTGCCCAATGCTT  400
  G  S  N  I  N  L   G  N  I  V  T  F  T   Q  F  V  S  V  T  L   I  Q  L  P  N  A    52

TGGACTTCTCTCACTTTCCG TTTAGGTTGCGACCTAGACA CATTCCTCTTAAGATCCATA TGTTAGCTGTGTTTTTGTTC  480
L  D  F  S  H  F  P   F  R  L  R  P  R  H   I  P  L  K  I  H   M  L  A  V  F  L  F   79

TTTACCAGTTCAGTCGCCAA TAACAGTGTGTTTAAATTTG ACATTTCCGTTCCGATTCAT ATTATCATTAGATGTTCAGG  560
 F  T  S  S  V  A  N  N  S  V  F  K  F   D  I  S  V  P  I  H   I  I  I  R  C  S  G  106

TACCACTTTGACGATGATAA TAGGTTGGGCTGTTTGTAAT AAGAGGTACTCCAAACTTCA GGTGCAATCTGCCATCATTA  640
  T  T  L  T  M  I   I  G  W  A  V  C  N   K  R  Y  S  K  L  Q   V  Q  S  A  I  I   132

TGACGCTTGGTGCGATTGTC GCATCATTATACCGTGACAA AGAATTTTCAATGGACAGTT TAAAGTTGAATACGGATTCA  720
M  T  L  G  A  I  V   A  S  L  Y  R  D  K   E  F  S  M  D  S   L  K  L  N  T  D  S   159

GTGGGTATGACCCAAAAATC TATGTTTGGTATCTTTGTTG TGCTAGTGGCCACTGCCTTG ATGTCATTGTTGTCGTTGCT  800
 V  G  M  T  Q  K  S   M  F  G  I  F  V   V  L  V  A  T  A  L   M  S  L  L  S  L  L  186

CAACGAATGGACGTATAACA AGTGCGGGAAACATTGGAAA GAAACTTTGTTCTATTCGCA TTTCTTGGCTCTACCGTTGT  880
  N  E  W  T  Y  N   K  C  G  K  H  W  K   E  T  L  F  Y  S  H   F  L  A  L  P  L   212

TTATGTTGGGGTACACAAGG CTCAGAGACGAATTCAGAGA CCTCTTAATTTCCTCAGACT CAATGGATATTCCTATTGTT  960
F  M  L  G  Y  T  R   L  R  D  E  F  R  D   L  L  I  S  S  D   S  M  D  I  P  I  V   239

AAATTACCAATTGCTACGAA ACTTTTCATGCTAATAGCTA ATAACGTGACCCAGTTCATT TGTATCAAAGGTGTTAACAT 1040
 K  L  P  I  A  T  K   L  F  M  L  I  A   N  N  V  T  Q  F  I   C  I  K  G  V  N  M  266

GCTAGCTAGTAACACGGATG CTTTGACACTTTCTGTCGTG CTTCTAGTGCGTAAATTTGT TAGTCTTTTACTCAGTGTCT 1120
  L  A  S  N  T  D   A  L  T  L  S  V  V   L  L  V  R  K  F  V   S  L  L  L  S  V   292
  ^.....................^.....................^.....................^
ACATCTACAAGAACGTCCTA TCCGTGACTGCATACCTAGG GACCATCACCGTGTTCCTGG GAGCTGGTTTGTATTCATAT 1200
Y  I  Y  K  N  V  L   S  V  T  A  Y  L  G   T  I  T  V  F  L   G  A  G  L  Y  S  Y   319

GGTTCGGTCAAAACTGCACT GCCTCGCTGAAACAATCCAC GTCTGTATGATACTCGTTTC AGAATTTTTCGGATTTTCTG 1280
 G  S  V  K  T  A  L   P  R  *                                                      328

CCGGATATGGTTTCTCATCT TTACAATCGCATTCTTAATT ATACCAGAACGTAATTCAAT GATCCCAGTGACTCGTAACT 1360

CTTATATGTCAATTTAAGCT                                                               1381
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FIG. 3. Nucleotide sequence and the predicted amino acid sequence of the Golgi membrane UDP-*N*-acetylglucosamine transporter of *K. lactis MNN2-2*. Depicted are the nucleotide sequence on the top and the amino acid sequence on the bottom. Nucleotide position 244 corresponds to the first ATG triplet coding for the initiator methionine. A consensus sequence for a TATA box toward the 5′ end of the nucleotide sequence is boxed in. The potential *N*-glycosylation sites are underlined. The leucine zipper motif is marked with a dotted line. The GenBank data base accession number is U48413.

transformed mutants to bind GS II lectin at the cell surface and the reaquisition of transport of UDP-GlcNAc into the lumen of Golgi vesicles. Both values were comparable to the corresponding ones of wild-type cells. Previously, biochemical analyses by Douglas and Ballou (5) had ruled out that the above mutant phenotype was the result of (*i*) differences in pool sizes of UDP-GlcNAc; (*ii*) quantitative and qualitative differences in endogenous mannan acceptors; (*iii*) differences in heat stability, apparent K_m values for substrates, metal requirements, and subcellular location of the terminal *N*-acetylglucosaminyl transferase; and (*iv*) the presence of an inactive enzyme precursor, a soluble inhibitor of the above transferase, or a hexosaminidase which removes terminal *N*-acetylglucosamine from mannoproteins. The multitransmembrane spanning protein is most likely a Golgi protein, since Golgi-enriched vesicles from wild-type and transformant *K. lactis* have such transport activity while vesicles from the above mutant do not. Outer mannan chains of *S. cerevisiae* are assembled in the Golgi lumen, and the terminal *N*-acetylglucosamine of *K. lactis* is added following the assembly of the mannans in the Golgi lumen.

Comparison of the 328-amino acid sequence of the *K. lactis* Golgi membrane UDP-GlcNAc transport depicted in Fig. 3 with sequences available in different data banks yielded two proteins with high degree of homology: a putative protein of *S. cerevisiae* that has an ORF of 342 amino acids and has 52% of identity and 71% of similarity over all the sequence, and a protein from *Caenorhabditis elegans* of 318 amino acids with 32% of identity and 52% of similarity over all the sequence.

The distribution of the hydrophobic segments of the *K. lactis* Golgi UDP-GlcNAc transporter is very similar to that of the other two homologs when compared by using the algorithm of Kyte and Doolittle (Fig. 4). A similar general conclusion was reached when the hydrophobic plots of the proteins were compared by the algorithm developed by Engelman *et al.* (20). The above results show that we are dealing with an integral membrane protein that contains multiple membrane spanning domains; however, fundamental questions regarding the secondary and three-dimentional structure of the *K. lactis* Golgi membrane UDP-GlcNAc transporter and its homologs remain to be determined. Different algorithms in computer programs such as PHDHTM (21) and MEMSTAT (22) predict that the above *K. lactis* Golgi membrane transporter may have between five and eight transmembrane domains. Without knowledge of the sidedness of the amino- and carboxy-terminal residues, we

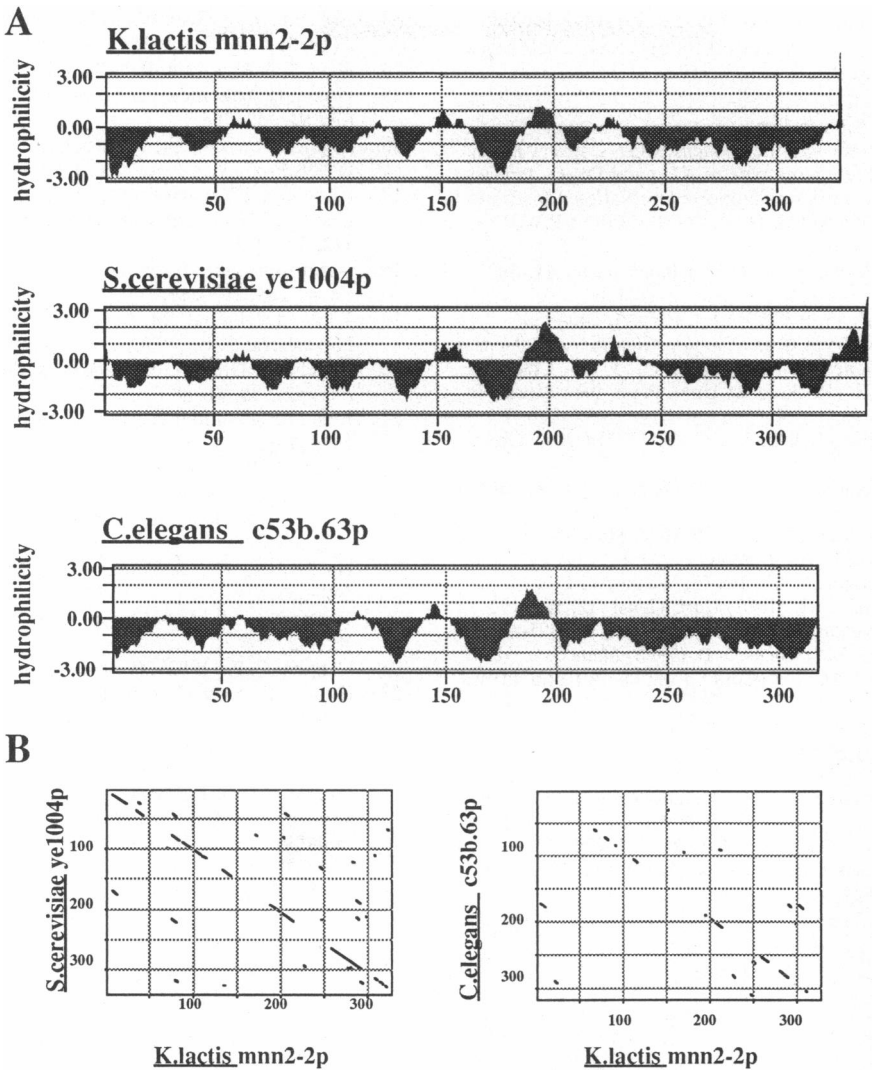

FIG. 4. (*A*) Hydrophobicity plots. Scale: Kyte-Doolittle (14), window size = 17. The *K. lactis* mnn2-2p has 328 amino acids (GenBank accession number U48413). The *S. cerevisiae* ye1004p has 342 amino acids (GenBank accession number U18530). The *C. elegans* c536.63p has 318 amino acids (GenBank accession number Z68215). (*B*) Diagonal matrix plots. Scoring matrix: pam 250; window size = 8; minimum percent score = 50; hash value = 2.

cannot predict whether the protein has even or odd numbers of transmembrane spanning domains. These predictions must also be considered very tentative until independent biochemical determination of the number of lumenal and cytosolic loops can be done. It is likely that the two putative glycosylation sites are not used, given their proximity to putative transmembrane helices. Because the antiporter of uridine diphosphate nucleotide sugars (UMP) is a competitive inhibitor of all uridine nucleotide sugar transporters, one would assume that there must be common structural recognition motifs facing both the cytosolic and lumenal domains of these transporters. Identification of these putative motifs is of importance.

To date, transporters from the Golgi apparatus membrane have not been cloned, although several transporters from the plasma and mitochondrial membrane have (23–27). Many of these latter proteins have multitransmembrane spanning domains, in many instances between 6 and 12 domains. The presence of a leucine zipper motif in the above UDP-GlcNAc transporter, by analogy to other membrane proteins, suggests that this domain may be involved in protein oligomerization (28, 29); this observation, together with recent studies suggesting that the rat liver Golgi membrane PAPS transporter is a homodimer (30), raises the possibility that the above UDP-GlcNAc transporter may also be a homodimer with multiple membrane spanning domains.

The protein sequence homology between the *K. lactis* Golgi transporter and that of putative proteins coded in *S. cerevisiae* and *C. elegans* of unknown function deserves some attention: *S. cerevisiae* does not have terminal *N*-acetylglucosamine residues in its glycoproteins, raising the question of the identity of the homologous transporter protein. The *S. cerevisiae* homolog does not correct the mnn2-2 phenotype of *K. lactis*. Analyses of an *S. cerevisiae* strain with the null mutation in the ye1004p ORF showed that the putative transporter is not required for cell viability.

The *K. lactis MNN2-1* and *MNN2-2* genes were thought to be allelic because they confer the same cell surface phenotype and are tightly linked (5). Now we know that they have a different biochemical defect: *MNN2-2* encodes the Golgi UDP-GlcNAc transporter that makes this nucleotide sugar available in the Golgi lumen as a substrate, together with mannoproteins, for the product of *MNN2-1* gene, which previously Ballou and coworkers (4) determined to be the α1–2-*N*-acetylglucosaminyl transferase. These two genes of *K. lactis* are not related and should not be confused with the *MNN2* gene of *S. cerevisiae*, whose function is not clear (31). The outer mannan chains of *S. cerevisiae* mnn2 cells only have unsubstituted α 1,6 linked mannoses, while *K. lactis* mnn2–1 and mnn2-2 cells do not lack any mannose in their outer chains.

Now that the protein sequence of a Golgi transporter is known, questions such as Golgi targeting determinants of

multitransmembrane spanning proteins, sub-Golgi localization, and interactions with corresponding glycosyltransferases can begin to be addressed.

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