The oligosaccharide profiles in glycoproteins are determined by a series of processing reactions catalyzed by Golgi glycosyltransferases and glycosidases. Recently in vivo galactose incorporation in Saccharomyces cerevisiae has been demonstrated through the expression of human β-1,4-galactosyltransferase in an alg1 mutant, suggesting the presence of a UDP-galactose transporter in S. cerevisiae (Schiwentek, T., Narimatsu, H., and Ernst, J. F. (1996) J. Biol. Chem. 271, 3398–3405). However, this is quite unexpected, because S. cerevisiae does not have galactose residues in its glycoproteins. To address this question we have constructed S. cerevisiae mnn1 mutant strains expressing Schizosaccharomyces pombe α-1,2-galactosyltransferase. The mnn1 mutant of S. cerevisiae provides endogenous acceptors for galactose transfer by the expressed α-1,2-galactosyltransferase. We present here three lines of evidences for the existence of UDP-galactose transporter in S. cerevisiae. (i) About 15–20% of the total transformed mnn1 cells grown in a galactose medium were stained with fluorescein isothiocyanate-conjugated α-galactose-specific lectin, indicating the presence of α-galactose residues on the cell surface. (ii) Galactomannan proteins can be precipitated with agarose-immobilized α-galactose-specific lectin from a whole cell lysate prepared from transformed mnn1 cells grown in a galactose medium. (iii) The presence of UDP-galactose transporter was demonstrated by direct transport assay. This transport in S. cerevisiae is dependent on time, temperature, and protein concentration and is inhibited by nucleotide monophosphate and Triton X-100. The overall UDP-galactose transport in S. cerevisiae is comparable with that in S. pombe, indicating a more or less similar reaction velocity, while the rate of GDP-mannose transport is higher in S. pombe than in S. cerevisiae.

N-Linked glycosylation is an essential modification that is highly conserved among all eukaryotic cells (1–4). The complex N-linked oligosaccharides have a wide variety of structure in animal cells, while they are relatively simpler in lower eukaryotes, such as Saccharomyces cerevisiae. However, in both cases the initial steps are nearly identical and involve the synthesis of Glc₃Man₉GlcNAc₂–PP-dol, transfer of oligosaccharide from lipid to protein, and subsequent trimming to Man₉GlcNAc₂ in the endoplasmic reticulum. In the latter stage S. cerevisiae elongates it to polymannose-type structure without adding any N-acetylglucosamine, galactose, and sialic acid residues that are characteristics of complex oligosaccharides in mammalian cells. Recently, interest in glycosyltransferases arose by their potential usefulness as tools for the synthesis of oligosaccharides in vitro (5) and for the remodeling of glycan chains of natural or recombinant glycoproteins. Yeast offers an attractive host for the production of heterologous proteins (6), and a number of recombinant glycoproteins were successfully produced in S. cerevisiae, although the sugars attached to proteins were confined to high mannose type. In this regard, the Δoch1Δmnn1 double mutant cells (7, 8) and Δoch1Δmnn1Δmnn4 triple mutant cells (9) are novel to produce glycoproteins containing N-linked core oligosaccharide Man₉GlcNAc₂ and to produce yeast specific acidic oligosaccharide (mannosylphosphorylated Man₉GlcNAc₂) content, respectively. In addition, the MNN6 gene encoding mannosylphosphate transferase is useful to produce mannosylphosphorylated Man₉GlcNAc₂ (10), which can be converted to phosphorylated Man₉GlcNAc₂ that functions as a sorting signal of lysosomal glycoproteins in mammalian cells (11), after releasing a terminal mannose residue. However, we are interested in further attempts to make an in vivo sugar structure remodeling toward the mammalian type structure in S. cerevisiae. To achieve this goal we need to express a number of cDNA encoding mammalian glycosyltransferases and specific sugar nucleotide transporters with an authentic localization having correct topology in the lumen of the Golgi in appropriate yeast mutant cells that provide specific endogenous acceptors for the expressed gene product (glycosyltransferase). As an essential step for the above goal, it is necessary to clone UDP-Gal transporter gene in S. cerevisiae. However, recently, the in vivo galactose addition was demonstrated by expressing the genes of mammalian β-1,4-galactosyltransferase (GalT) in S. cerevisiae (alg1 mutant), which suggests the presence of unexpected UDP-Gal transporter, allowing β-1,4-GalT to act inside the lumen of the Golgi to add galactose onto endogenous acceptors under nonpermissive growth conditions (11). According to the hypothesis of a sugar nucleotide transport system in the Golgi apparatus, an antiporter for the corresponding sugar nucleotide must be present in the lumen of the Golgi apparatus, and in the case of UDP-Gal transport, UMP must be present to act as an antiporter (12). A galactosyltransferase inside the lumen is necessary to transfer galactosyltransferase.
ose from UDP-Gal to endogenous acceptors to produce UDP-Gal, which can be subsequently hydrolyzed to UMP by a UDPase. Since the presence of a UDPase is demonstrated in S. cerevisiae by the presence of UDP-Gal transport system in S. cerevisiae by a direct transport assay. It is quite surprising to us that S. cerevisiae has UDP-Gal transport activity without any physiological need. At present it remains unsolved without further evidence that this transport activity is from a specific UDP-Gal transport or other sugar nucleotide transport having a broader substrate specificity. However, this finding is promising for the achievement of our goal for the incorporation of galactose residues into sugar chain of the glycoproteins in S. cerevisiae, when the appropriate acceptor glycans are provided.

**EXPERIMENTAL PROCEDURES**

**Materials**—Materials were obtained as follows. UDP-Gal, GDP-Man, Hesperidin, concanavalin A-androgenin, Dowex-1X8-400 (CI-), were from Sigma Chemical Co. (St. Louis, MO). N-acetylmannosamine (500 mM) was from Biochrom (Cambridge, U.K.). FITC were from Vector Laboratories (Burlingame, CA). CNBr-Sepharose 4B was from Pharmacia Biotech Inc. GDP-[3H]-mannose (25 mCi/mmol) was from NEN Life Science Products. UDP-[3H]galactose (7.9 Ci/mmol) and carrier-free Na2[32P]PO4 were obtained from Amersham Corp. Zymolyase-1010T (Kiryu Brewery Co.) was obtained from Seikagaku Kogyo Co. Ltd. (Tokyo, Japan) and “complete” (protease inhibitor mixture tablets) from Boehringer Mannheim GmbH, Mannheim, Germany. IODO-BEADs and a BCA protein assay kit were obtained from Pierce. Filters and filtration apparatus were from Millipore Corp. (Bedford, MA). UDP, UMP, GDP, and GMP were from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Clear-sol scintillation mixture was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). All other reagents were of the highest purity commercially available.

**Strains, Media, and Genetic Methods**—The S. cerevisiae strains used in this work are RA1-1B (MATa leu2 ura3 trpl ade8 his3) (9), LB1-3B (MATa mnn2 SUC2 mal gal2 CUP1) (14), and R16B (MATa mnn1 leu2 ura3 trpl ade6 his1 and/or his3 lys2). The last strain was a segregant of diploid between RA1-1B and YNS3-7A (MATa och1::LEU2 mnn1 leu2 ura3 his1 and/or his3) (2). The S. pombe strain used in this work is YJ741 (h ura4 leu6 ade6-M216) (15). The media for culture of S. pombe were swollen for 1 h and washed on a sintered glass (G3) with 1 mM EDTA. The membrane pellet was resuspended in TMS buffer to a protein concentration of 25 µg/ml of given sugar acceptor. Reaction mixture contained 0.1–0.2% (v/v) Triton X-100, depending on the concentration in the enzyme fraction. After incubation for 30 min at 30 °C, 1% of added UDP-[3H]Gal with 5 µmol of given sugar acceptor. Reaction mixture contained 0.1–0.2% (v/v) Triton X-100, depending on the concentration in the enzyme fraction. After incubation for 30 min at 30 °C, the reaction was terminated by adding 200 µl of ice-cold water and loaded onto a 1.0 ml of Dowex-1 (Cl form, 200–400 mesh) anion exchange column packed in a 3-ml syringe. The column was washed twice with 1.0 ml of water and the combined eluents were mixed with 25 volumes of scintillation mixture. The Dowex columns were regenerated by washing 2.5 ml of 5 M NaCl, 2.5 ml of 0.1 M HCl, and then 8.0 ml of water. One unit of enzyme activity is defined as the amount of enzyme that catalyzes the transfer of 1.0 nmol of galactose from UDP-Gal to endogenous acceptor.

**GDPase Assay**—The assay was done essentially as described by Abejon et al. (23). Briefly, incubation mixture in a final volume of 0.1 ml contained enzyme (20 µg of Triton X-100 solubilized Pfraction) (see“Subcellular Fractionations”), CaCl2 (1 mM), Triton X-100 (100 µg), GDP (0.2 µM), and imimidazole buffer, pH 7.6 (20 mM). Incubation was performed in a water bath at 37 °C for 1 h. The reaction was stopped by adding 10 µl of 10% (w/v) SDS. The released inorganic phosphate was determined by the Ames (24) method. The absorbance was measured at 820 nm and the amount of inorganic phosphate released was calculated from a calibration curve prepared by using KH2PO4 as a standard. One unit of activity was defined as 1 µmol of inorganic phosphate released per min under standard assay conditions. Specific activity is calculated as units/mg of protein. Latency of GDPase was calculated according to Abejon et al. (23).

**Assay of Marker Enzymes**—The endoplasmic reticulum marker enzyme NADPH-dependent cytochrome c reductase was measured as described previously (25), and cytochrome c oxide (mitochondrial marker enzyme) (26) and α-mannosidase (vacuolar marker enzyme) (27) were assayed as described.

**Protein Quantitation**—The protein concentration was measured by BCA reagent (Pierce) using bovine serum albumin as a standard.

**Preparation of G. simplificola I-B4-agarose**—GS I-B4 was coupled with CNBr activated Sepharose 4B according to Roy et al. (28) with some modifications. Five hundred milligrams of CNBr-Sepharose 4B were swollen for 1 h and washed with a sintered glass (G3) with 1 mM HCl. Five hundred milligrams of pure lecin (GS I-B4) were used in NaHCO3 buffer (10 mM, pH 8.3) containing NaCl (0.5 mM), mixed with the gel, and kept overnight at 4 °C. For blocking the remaining active groups, the gel was transferred to blocking agent, 0.2 M glycine (pH 8.0). To wash away excess unbound adsorbed proteins, the gel was alternatively washed three or four times with high pH coupling buffer (pH 8.3) and then low pH Na-acetate buffer (pH 4.2), each containing 0.5 mM NaCl. This ensured that no free ligand remained ionically bound to the immobilized ligand.

**Subcellular Fractionations**—The Golgi-rich fractions were isolated as described by Walworth and Novick (29) with some modifications. Briefly, cells were grown at 30 °C in a chemically defined medium supplemented with 50 mM potassium phosphate, pH 7.5, 10 mM NaNO3, 40 mM 2-mercaptoethanol, and yamazymase 100T, 1

2 Y. Shimma, unpublished results.
mg/g of wet cells). The cell suspension in spheroplastic buffer was incubated at 37 °C for 45 min with occasional gentle mixing. The spheroplasts were pelleted in a table top centrifuge (Beckman, GS 6KR), and washed twice with 1.0  1 ice-cold sorbitol to remove zymolyase. The cells were then suspended in ice-cold lysis buffer (0.8  1 Sorbitol in 10 mM tris(hydroxymethyl)aminomethane, pH 7.2, and anti-protease mixture). The spheroplasts were transferred to a 50-ml Dounce homogenizer (Wheaton Scientific, Millville, NJ) on ice and lysed with 20 strokes of a Teflon pestle. Low speed centrifugation at 1,000  1 g for 10 min yielded a large pellet containing unlysed cells and cell wall debris. The post 1,000  1 g supernatant (S1) was carefully collected and then centrifuged at 10,000  1 g for 1 h at 4 °C (Hitachi, Himac SCR20B, Japan), which yielded a pellet (P1) of nuclear and mitochondrial fraction. The post mitochondrial supernatant (S2) was then centrifuged at 100,000  1 g (Beckman ultracentrifuge L-80 Optima, in 70.1TI rotor) and yielded a pellet (P2) of Golgi-enriched fraction and the cytosolic supernatant (S3).

Isolation of Total Cellular Proteins—Yeast cells were grown in an appropriate medium and harvested at 3,000 rpm and washed with 10 mM NaN3, and 10 mM dithiothreitol, suspended in LIP buffer (50 mM Tris-Cl, pH 7.4, 1% Triton X-100, 150 mM NaCl, 5 mM EDTA). Cells were disrupted by glass beads by vortexing (at full power 4 °C) for 1 h. The precipitated proteins were isolated by centrifugation at 10,000  1 g for 45 min at 4 °C, washed twice with cold acetone, and reisolated by centrifugation. Isolated proteins were dissolved in iodination buffer (100 mM Na-phosphate buffer, pH 7.0).

Radioiodination of Whole Cell Proteins—Proteins were iodinated by IODO-BEADS according to the manufacturer’s instructions. The iodination reaction was started by adding Na125I (200 Ci) and two IODO-BEADS to 500 µg of protein, dialyzed, and dissolved in 0.1 X Na-phosphate buffer, pH 7.0. The reaction mixture was incubated at room temperature for 10 min. The iodination reaction was terminated by removal of IODO-BEADS, and the iodinated proteins were dialyzed extensively against water and then PBS to remove free iodine.

Gel Electrophoresis—SDS-polyacrylamide gel electrophoresis (PAGE) was conducted under reducing conditions as described previously (30). Gels were run in the presence of precasted SDS-PAGE standards (Bio-Rad): myosin (M, 199,000),  b-galactosidase (M, 120,000), bovine serum albumin (M, 67,000), and ovalbumin (M, 48,000).

Lectin Precipitation—Lectin precipitation was performed according to Schwientek et al. (11). In short, 25 µl of 30% (w/v) concanavalin A-agarose 4B and 35 µl of 50% (w/v) of GS I-B4-agarose was added to 100 µg of iodinated whole cell proteins in PBS to precipitate manno-proteins and galactomannoproteins, respectively. The precipitated pellet was washed twice with PBS, solubilized with Laemmili (31) solubilization buffer, boiled for 15 min, and subjected to 10% SDS-PAGE. The gels were fixed, dried, and exposed to Kodak X-Omat film at –70 °C for 12 h.

Lectin Staining of Cells—Yeast cells were stained with FITC-conjugated GS I-B4 as follows. Freshly grown cells (2  108) of various strains were washed in PBS and taken in 50 µl of PBS. GS I-B4-FITC (2.5 µl, 1.0 mg/ml) was added and incubated on ice for 10 min in dark. After incubation the cells were washed three times with 200 µl of PBS, resuspended in 10–15 µl of PBS, and observed under fluorescence microscope (Olympus BX50, Japan).

FACS Analysis—The samples were prepared same as described above using same lectin. The cells were sorted in a fluorescent cell sorter (Becton Dickinson FACS Calibur). The positive cells were isolated and concentrated in a “built-in” cell concentrator.

Isolation of Yeast Mannans—Mannans were isolated according to Peat et al. (32) with modifications. Freshly grown yeast cells were isolated by centrifugation, washed with 1% (w/v) KCl, and resuspended in 20 mM citrate buffer (pH 7.0). The cell suspension was then heated in an autoclave at 120 °C for 1 h. Supernatant was isolated by centrifugation, and 2 volumes of ethanol were added. The crude mannan was precipitated by centrifugation (10,000  1 g for 20 min). The precipitated mannans was then dissolved and dialyzed against distilled water with two changes. Protein concentrations were estimated and used as acceptor substrates for galactosyltransferase assay.

UDP-Galactose Transporter in S. cerevisiae

**TABLE I**

| Enzyme Source | Galactosyltransferase Activity (nmol/min/mg protein) |
|---------------|--------------------------------------------------|
| **With acceptor** | **Without acceptor** |
| JY741* | R16B* | R16B (pKT10) | R16B (YEpUGAP-gma12") | 10.87 |
| 2.80 | 0.30 | 0.21 | 0.51 |

* Enzyme source: Triton X-100-solubilized microsomal fraction prepared from different cells as described under “Experimental Procedures.”

* Sugar acceptor:  a-methyl 1-mannoside (5 µmol/50 µl of reaction mixture).

* S. pombe wild-type strain.

* S. cerevisiae mnn1 strain.

mat MgCl2) and placed on ice. Diluted samples were then applied onto filtration apparatus containing HA filters (24 mm diameter, 0.45-µm pore size). The filters were washed with another 10 ml of wash buffer. The filters were air-dried and placed in 15-mL counting vials, and 2 ml of ethylene glycol methyl ether were added. The vials were allowed to stand at room temperature with occasional shaking until the filters were dissolved (about 30 min). Ten milliliters of scintillation mixture (Clearsol) were added, and the samples were counted in a liquid scintillation counter (Beckman LS1701). The amount of radioactivity that was bound nonspecifically to the outside of the vesicles was determined by zero time assay.

RESULTS

Expression of S. pombe a-1,2-Galactosyltransferase gene in S. cerevisiae—gma12 * gene encoding a-1,2-GalT of S. pombe was expressed in S. cerevisiae cells. Plasmid YEpUGAP-gma12 * carries gma12 * gene under the control of the S. cerevisiae TDH3 promoter. YEpUGAP-gma12 * and control plasmid pKT10 were introduced into S. cerevisiae R16B mnn1 mutant cells (the choice of this mutant is further described under “Discussion”). GalT activity in solubilized microsomal fractions from these transformant cells was assayed using a-1-methyl a-mannoside as an acceptor substrate (Table I). The enzyme preparation from transformed cells expressing gma12 * gene showed a higher specific activity toward galactose transfer than the control counterpart cells. This GalT activity in transformed cells is acceptor dependent (Table I).

Since the full-length gma12 * gene includes sequences coding for both transmembrane and cytoplasmic domains (20), the expressed enzyme is expected to be localized in the Golgi vesicles with the same topography as in S. pombe. As shown in Table IIA, a considerable amount of GalT activity is associated with the low speed pellet (P2) and about 34% of total enzyme activity is associated with the 100,000  1 g (P3), where as about 52% of GDPase activity (Golgi marker), 16% of a-mannosidase (vacuolar marker), 18% of NADPH-dependent cytochrome c reductase (endoplasmic reticulum marker) and 24% of cytochrome c oxidase (mitochondrial marker) are associated with vesicle fraction (P3). However, the relative distribution of above proteins on the basis of specific activity indicated that the enzyme (a-1,2-GalT) was highly abundant in the vesicle fraction (P3) with an overall increase in specific activity of 4.9-fold (Table IIB, 5.43 in P2 vs. 1.1 in P3). In this study the specific activity of GDPase has increased about 5.3-fold, whereas the specific activities of marker enzymes of other organelles such as NADPH-dependent cytochrome c reductase, cytochrome c oxidase, and a-mannosidase has reduced by 2.4, 9.8-, and 5.5-fold, respectively. Regarding the topology of high speed pellet (P3) associated enzymes about 70% of the GalT has...
UDP-Galactose Transporter in S. cerevisiae

**TABLE II**

Localization and topology of expressed S. pombe α-1,2-galactosyltransferase in S. cerevisiae

| Enzyme | Activity in different subcellular fractions* |
|--------|---------------------------------------------|
|        | S1 | S2 | P2 | S3 | P3 |
| α-1,2-Galactosyltransferase | 100 | 95 | 55 | 45 | 21 |
| GDPase | 100 | 95 | 65 | 35 | 13 |
| α-Mannosidase | 100 | 90 | 70 | 30 | 16 |
| NADPH cytochrome c reductase | 100 | 90 | 30 | 70 | 12 |
| Cytochrome c oxidase | 100 | 90 | 34 | 66 | 10 |

| Enzyme | Specific activity |
|--------|-------------------|
|        | units/mg protein |
| α-1,2-Galactosyltransferase | 1.10 | 1.95 | 0.85 | 1.30 | 5.43 |
| GDPase | 0.23 | 0.62 | 0.25 | 0.06 | 1.21 |
| α-Mannosidase | 0.11 | 0.13 | 0.12 | 0.06 | 0.02 |
| NADPH cytochrome c reductase | 24.0 | 4.0 | 102.0 | 2.8 | 10.0 |
| Cytochrome c oxidase | 7.1 | 1.48 | 11.7 | 0.21 | 0.73 |

| Enzyme | Activity* |
|--------|-----------|
|        | No Triton X-100 | Triton X-100 (0.1% v/v) |
| α-1,2-Galactosyltransferase | 31 | 69 |
| GDPase | 5 | 95 |

*Total activities (units) present in 1000 × g supernatant (S1) were taken as 100%. Units of enzyme activity are expressed as follows. α-1,2-GaT, 1.0 nmol of galactose transferred to α-methyl β-mannoside per min; GDPase, 1.0 µmol P1 released from GDP per min; α-mannosidase, µmol p-nitrophenyl-α-D-mannopyranoside hydrolyzed per 30 min; cytochrome c oxidase, K (first order rate constant) per min; NADPH cytochrome c reductase, 1.0 µmol of cytochrome c reduced per min.

Enzyme activities were determined under standard assay conditions as described under "Experimental Procedures" using P3 fraction as enzyme source with or without Triton X-100. 100% activity corresponds to the sum of activities in presence and absence of Triton X-100.

**TABLE III**

Acceptor specificity for transferase reactions

| Acceptor | Activity % |
|----------|------------|
| A. Sugar acceptor specificity of expressed α-1,2-galactosyltransferase | 100.0 |
| α-Methyl β-mannoside | 100.0 |
| α-Methyl N-galactoside | 5.0 |
| D-Mannose | 32.0 |
| D-Galactose | 5.2 |
| Mannotriose (α-1,2 linked) | 18.0 |
| N-Acetyl D-glucosamine | 5.0 |
| None | 4.8 |

B. Crude mannoproteins (mannan) as acceptors for expressed α-1,2-galactosyltransferase isolated from different mutant strains of S. cerevisiae

| Malignant | Activity % |
|----------|------------|
| WT (RA1–1B) | 100.0 |
| mnn1 (R16B) | 373.3 |
| mnn2 (L1B–3B) | 81.7 |
| Albumin | 30.9 |
| None | 28.8 |

*Values were expressed as the percent of the activity obtained with α-methyl β-mannoside as an acceptor which was taken as 100%. Enzyme was assayed as described under "Experimental Procedures." One hundred percent activity corresponds to 10.5 units/mg protein.

Values obtained with the crude mannoprotein isolated from RA1–1B strain was take as 100%. Enzyme was assayed as described under "Experimental Procedures" except that mannans were used as acceptors. In each assay 0.5 mg (0.142 mM) mannan was used 100% activity corresponds to 1.8 units/mg protein. The molar concentration was calculated on the basis of the average molecular weight (70 kDa) of mannan.

*Numbers shown are percentage of control activity.

Lectin Precipitation—To further confirm in vivo galactose incorporation, we have tried to precipitate proteins containing specific sugars by using sugar- and linkage-specific lectins from whole cell lysates. The total cellular proteins were isolated from yeast cells and iodinated as described under "Experimental Procedures." A number of bands were detected when con-

canavalin A-agarose was used to precipitate mannoproteins from whole cell proteins prepared from S. pombe and different transformants of S. cerevisiae either carrying gma12 expressing or control plasmids (Fig. 2A, lanes 1–5). In contrast, when GS I-B4-agarose was used to precipitate galactomannoproteins, only cellular proteins from S. pombe and S. cerevisiae
GDP-Man and UDP-Gal transport in S. pombe—GDP-Man and UDP-Gal transport is saturable in vesicles derived from S. pombe, as shown in Figs. 5A, and 6A. Although in both cases the transport is saturable, the rate of GDP-Man transport is much higher than that of UDP-Gal under the same substrate concentration. The $V_{\text{max}}$ of GDP-Man and UDP-Gal are 420 pmol/mg/5 min and 16 pmol/mg/5 min, respectively. The apparent $K_m$ of GDP-Man and UDP-Gal transport are 3.5 $\mu$M and 6.7 $\mu$M, respectively. The rate of UDP-Gal transport is also dependent on the incubation temperature (the rate of transport at 4 °C is about 42% compared with that obtained at 30 °C) and inhibited in the presence of Triton X-100, specific sugar nucleotides and nucleotide di/monophosphate (Table IV).

GDP-Man and UDP-Gal transport in S. cerevisiae—Both GDP-Man and UDP-Gal transports are also saturable in vesicles derived from S. cerevisiae (Figs. 5B and 6B) and likewise, the rate of GDP-Man transport is much higher than that of UDP-Gal transport. The $V_{\text{max}}$ of GDP-Man and UDP-Gal are 123 pmol/mg/5 min and 13 pmol/mg/5 min, respectively. The apparent $K_m$ values of GDP-Man and UDP-Gal transport are 2.5 and 5.0 $\mu$M, respectively. UDP-Gal transport in S. cerevisiae is time and protein concentration dependent (Fig. 4, A and B). The transport rate is temperature dependent (the rate of transport at 4 °C is about 40% compared with that obtained at 30 °C) and inhibited in the presence of Triton X-100. Transport is inhibited in the presence of specific sugar nucleotide and nucleotide di/monophosphates (Table IV).

**DISCUSSION**

Glycosyltransferases are responsible for glycoprotein biosynthesis, by transferring sugar residues from a nucleotide sugar to a growing sugar chain, and are resident membrane proteins of the endoplasmic reticulum and Golgi apparatus. The specificity of these transferases for their donor and acceptor substrates constitutes and determines the primary structures of the sugar chains produced by the cell. The glycosylation pathways in eukaryotes from yeast to mammals have been extensively studied and reviewed (1–4). It has been reported that mammalian glycosyltransferases can be produced in S. cerevisiae in an active form (35, 36), even though they do not have any influence on the host sugar structure. However, recently, the in vivo galactose addition in S. cerevisiae has been demonstrated by expressing human β-1,4-GalT in the alg1 mutant (11). In their report some evidences were presented that suggest the presence of UDP-Gal transporter in S. cerevisiae.

These indirect but strong evidences stimulated us as an attractive proposition to check the presence of a UDP-Gal transport system in S. cerevisiae by direct transport assay, because this point is critical when we consider a sugar structure remodeling toward mammalian complex type oligosaccharides by using yeast cells. The alg1 mutant combined with the expression of β-1,4-GalT is not suitable as far as to study a stable in vivo galactose addition in S. cerevisiae, because the alg1 mutant offers acceptors for galactose transfer only at the nonpermissive temperature. We have chosen mnn1 mutant of S. cerevisiae combined with the expression of S. pombe α-1,2-GalT, which is capable of transferring galactose to a variety of mannose acceptors under the growing conditions (22), because galactose addition sites so far studied (37, 38) include terminal Gal-α-1,2-Man residue, that corresponds to terminal Man-α-1,3-Man residues in S. cerevisiae. The recombinant α-1,2-GalT is enzymatically active and displays similar characteristics for acceptor substrate specificity as reported in S. pombe (Table I).

Mannoproteins isolated from mnn1 cells act as a better accept-
tor than those from wild type cells or mnn2 mutant cells (Table II), which may be reasonable when considering the structural similarity between proposed galactomannan structures in *S. pombe* (37, 38) and mannan structures in *mnn1* mutant (14), both of which contain a common α-1,2-linked mannobiose or mannotriose structure that can serve as an acceptor. The results of this study bear out validity of the hypothesis. We were able to stain 15–20% of *mnn1* cells expressing *S. pombe* α-1,2-GalT with FITC-conjugated α-galactose-specific lectin GS I-B4, only when the cells were grown in a synthetic medium using galactose as a sole carbon source (Fig. 1). We were unsuccessful to concentrate those stained cells by FACS sorting to increase the percentage of stained cells. Although the sorted positive cells were regrown in a galactose medium followed by lectin staining, the percentage of the stained cells remained unchanged. This partial staining may be partly due to the limitation of acceptor recognition by recombinant α-1,2-

![Fig. 2](http://www.jbc.org/)

**Fig. 2.** Autoradiograms of SDS-PAGE (10%) of lectin-agarose-precipitated proteins from whole cell lysates that were radiolabeled with Na125I. Radiolabeling procedure was as described under “Experimental Procedures.” Concanavalin A-agarose (panel A) and GS I-B4-agarose (panel B) were used for the lectin precipitation. The cell lysates were prepared from the following cells: lane 1, *S. pombe* grown in YPD; lane 2, *S. cerevisiae* mnn1 (YEpUGAPgma12′) cells grown in SCGal-Ura; lane 3, *S. cerevisiae* mnn1 (YEpUGAPgma12′) cells grown in SC-Ura; lane 4, *S. cerevisiae* mnn1 (pRT10) cells grown in SCGal-Ura; lane 5, *S. cerevisiae* mnn1 grown in YPD.

![Fig. 3](http://www.jbc.org/)

**Fig. 3.** Time course and protein concentration dependence of UDP-Gal transport in *S. pombe*. AP3 vesicle fraction was incubated with 10 μM UDP-[3H]Gal (360 cpm/pmol) at 30 °C in a final volume of 300 μl. After incubation the transport activity was measured as described under “Experimental Procedures.” Panel A, dependence for incubation time, for which 0.5 mg of vesicle protein per assay was used. Panel B, dependence for protein concentration, for which a 5-min incubation was used. The results shown are the mean of two separate determinations.

![Fig. 4](http://www.jbc.org/)

**Fig. 4.** Time course and protein concentration dependence of UDP-Gal transport in *S. cerevisiae*. A P3 vesicle fraction was incubated with 10 μM UDP-[3H]Gal (360 cpm/pmol) at 30 °C in a final volume of 300 μl. After incubation the transport activity was measured as described under “Experimental Procedures.” Panel A, dependence for incubation time, for which 0.5 mg of vesicle protein per assay was used. Panel B, dependence for protein concentration, for which a 5-min incubation was used. The results shown are the mean of two separate determinations.

UDP-Galactose Transporter in *S. cerevisiae*

GalT, because more than one Gal-Ts are present in *S. pombe* (20), and also partly due to the limitation of accessibility (low affinity) of endogenous acceptor toward α-1,2-GalT. However, the latter case seems unlikely because the 15–20% staining is reproducible in any transformants in a growth phase independent manner, suggesting that the putative changes of acceptor mannans during the cell growth may not affect the affinity toward α-1,2-GalT in *S. cerevisiae*. In support of this, the precipitation of galactomannan by GS I-B4-agarose (Fig. 2) demonstrates the in vivo galactose addition not only to the cell surface but also to the total cellular glycoproteins. Similar phenomena of partial cell surface staining (30–50% of total cells) with FITC-conjugated specific lectins were also observed in Chinese hamster ovary cells transfected with α-1,2-fucosyltransferase cDNA (39). At this point it is difficult to speculate the actual limiting factor for the staining without further study. *S. pombe* has a physiological demand for a transport of
The presence of UDP-Gal transporter is quite unexpected for *S. cerevisiae* because *S. cerevisiae* does not add any galactose to its proteins. Our present results on UDP-Gal transport in *S. cerevisiae* are comparable with those in *S. pombe* and in *S. cerevisiae* mutants, which accumulate intermediate glycan chains that may serve as an endogenous acceptor for galactose transfer (11), were used as host cells. Therefore, the lack of UMP may inhibit the total transport activity even in the presence of a functional transporter.

In this report we have provided direct evidence for the presence of a UDP-Gal transport system in *S. cerevisiae*. The rate of UDP-Gal transport in *S. cerevisiae* is comparable with that in *S. pombe*, while the rate of GDP-Man transport in *S. pombe* is much higher than that in *S. cerevisiae*. Several lines of evidence have excluded the possibility that the activity of a UDP-Gal transporter in *S. cerevisiae* may be derived from a non-specific binding of UDP-[3H]Gal to the surface of Golgi vesicles. If such counts are originated from non-specific binding, one might expect that there may not be any changes in counts in both incubation time and incubation temperature independent manners, which is absolutely inconsistent with our results (Figs. 3 and 4). The calculated apparent $K_m$ for GDP-Man transport in *S. cerevisiae* is 2.5 $\mu$m, which is comparable with the published results, the apparent $K_m$ was reported 2.0 $\mu$m and 3.0 $\mu$m (23, 34). The apparent $K_m$ for UDP-Gal transport for both *S. pombe* and *S. cerevisiae* are with in the expected values for sugar nucleotide transport (12).

**Table IV**

| Addition | Concentration | Inhibition of transport* | S. pombe | S. cerevisiae |
|----------|---------------|--------------------------|----------|--------------|
|          | $\mu$m        | %                        |          |              |
| Complete |               | 0.0                       | 0.0      |              |
| UMP      | 10            | 34.0                      | 48.3     |              |
| UDP      | 10            | 25.5                      | 36.8     |              |
| UDP-Glc  | 10            | 21.0                      | 27.0     |              |
| UDP-Gal  | 10            | 47.0*                     | 45.1*    |              |
| GDP-Man  | 10            | 21.5                      | 17.7     |              |
| Triton X-100 | 0.1%(v/v) | 94.0                      | 92.0     |              |

* One hundred percent activity corresponds to 16 pmol/mg/5 min and 13 pmol/mg/5 min, respectively, for *S. pombe* and *S. cerevisiae*.
* This is not due to an inhibition, but due to a titration by an equal amount of hot and cold UDP-Gal.

GDP-Man and UDP-Gal in the Golgi vesicles, while the presence of UDP-Gal transporter is quite unexpected for *S. cerevisiae* because *S. cerevisiae* does not add any galactose to its proteins. Our present results on UDP-Gal transport in *S. cerevisiae*, along with the previous report (11), strongly demonstrate the presence of a UDP-Gal transport system in *S. cerevisiae*. However, recently, inconsistent results have been presented reporting no detectable UDP-Gal transporter activity in *S. cerevisiae* (40). We believe that their failure to detect any UDP-Gal transporter activity is due to the absence of UMP generating system in their transport assay system. According to the theory (34, 41), sugar nucleotide transport is dependent on the antiporter concentration in the lumen of the Golgi. In their case, even though cells were grown in a galactose medium to drive the gene expression under the control of GAL1 promoter that will also provide UDP-Gal in the cytosol (11), no GalTs were expressed to transfer galactose from UDP-Gal to endogenous acceptors and to produce UDP, which should be in turn hydrolyzed to UMP by a UDPase that is known to be present in the lumen of the Golgi of *S. cerevisiae* (13). In addition, no appropriate *S. cerevisiae* mutants, which accumulate intermediate glycan chains that may serve as an endogenous acceptor for galactose transfer (11), were used as host cells. Therefore, the lack of UMP may inhibit the total transport activity even in the presence of a functional transporter.

In this report we have provided direct evidence for the presence of a UDP-Gal transport system in *S. cerevisiae*. The rate of UDP-Gal transport in *S. cerevisiae* is comparable with that in *S. pombe*, while the rate of GDP-Man transport in *S. pombe* is much higher than that in *S. cerevisiae*. Several lines of evidence have excluded the possibility that the activity of a UDP-Gal transporter in *S. cerevisiae* may be derived from a non-specific binding of UDP-[3H]Gal to the surface of Golgi vesicles. If such counts are originated from non-specific binding, one might expect that there may not be any changes in counts in both incubation time and incubation temperature independent manners, which is absolutely inconsistent with our results (Figs. 3 and 4). The calculated apparent $K_m$ for GDP-Man transport in *S. cerevisiae* is 2.5 $\mu$m, which is comparable with the published results, the apparent $K_m$ was reported 2.0 $\mu$m and 3.0 $\mu$m (23, 34). The apparent $K_m$ for UDP-Gal transport for both *S. pombe* and *S. cerevisiae* are with in the expected values for sugar nucleotide transport (12). The overall profile of UDP-Gal transport is similar in *S. pombe* and *S. cerevisiae* (Fig. 6) with little difference in apparent $K_m$, which are 6.7 and 5.0 $\mu$m, respectively. The inhibition study of UDP-Gal transport by nucleotide diphosphates and sugar nucleotides, together with the loss of counts with the treatment of Triton X-100, provides the reliability of our results, indicating that the inhibition by UMP and UDP (36.8–48.3 %) is much higher than that by GDP-Man (17.7 %) (Table IV).

Although the rate of UDP-Gal transport in both yeast species are comparable, it is still difficult to speculate the physiological role of UDP-Gal transporter in *S. cerevisiae*. It remains to be elucidated that this activity may be derived from other sugar nucleotide transporters with a broader substrate specificity, for instance, UDP-glucose transporter, which is necessary for
β-1,6-glucan synthesis along the secretory pathway (42). We made an attempt to throw some light by inhibition study of UDP-Gal transporter by cold UDP-Glc, which showed about 21 and 27% inhibition for \textit{S. pombe} and \textit{S. cerevisiae}, respectively (Table IV). However, from these data it is difficult to predict something, because a wide variation of inhibition of UDP-GlcNAc transporter by different UDP-sugar nucleotides has been reported (43), suggesting all UDP-sugar nucleotides has different affinity for the active site of transporter. 

Acknowledgments—We are indebted to Y. Shimma and T. Odani for providing the R16B strain.
Functional Evidence for UDP-galactose Transporter in *Saccharomyces cerevisiae* through the *in Vivo* Galactosylation and *in Vitro* Transport Assay
Samir Kumar Roy, Takehiko Yoko-o, Hiroshi Ikenaga and Yoshifumi Jigami

*J. Biol. Chem.* 1998, 273:2583-2590.  
doi: 10.1074/jbc.273.5.2583

Access the most updated version of this article at [http://www.jbc.org/content/273/5/2583](http://www.jbc.org/content/273/5/2583)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 36 references, 19 of which can be accessed free at [http://www.jbc.org/content/273/5/2583.full.html#ref-list-1](http://www.jbc.org/content/273/5/2583.full.html#ref-list-1)