BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **232,** 121–125 (1997)
ARTICLE NO. RC976239

The *Schizosaccharomyces pombe gms1*$^+$ Gene Encodes an UDP-Galactose Transporter Homologue Required for Protein Galactosylation

Mitsuaki Tabuchi, Naotaka Tanaka, Shojiro Iwahara, and Kaoru Takegawa[1]
Department of Bioresource Science, Faculty of Agriculture, Kagawa University, Miki-cho, Kagawa 761-07, Japan

Received January 28, 1997

In a previous study, we isolated a *Schizosaccharomyces pombe* mutant defective in protein galactosylation (Takegawa, K., Tanaka, N., Tabuchi, M. and Iwahara, S. (1996) *Biosci. Biochem. Biotech.* 60, 1156–1159). From an *S. pombe* genomic library, we cloned the *gms1*$^+$ gene which restored the galactosylation of cell wall glycoproteins. Gms1 protein shares significant sequence similarity with human UDP-galactose and murine CMP-sialic acid transporters. The fission yeast strains deleted for the *gms1*$^+$ gene lacked galactose residues in sell surface glycoproteins and were significantly decreased in UDP-galactose transport activity. These results showed that the *gms1*$^+$ encodes an UDP-galactose transporter, and this protein appears to be an essential role for the incorporation of UDP-galactose into the lumen of Golgi in *S. pombe*. © 1997 Academic Press

In the budding yeast *Saccharomyces cerevisiae,* elongation of the N-linked oligosaccharide occurs by the extension of α1,6-linked mannose backbone onto which are built side chains containing α1,2- and α1,3-linked mannose residues (1). Many glycosylation defective mutants have been isolated from *S. cerevisiae,* and genetic selections have resulted in the identification of many genes required for the glycosylation event (2). Molecular characterization of these gene products has revealed a number of biochemical activities involved in this process. In contrast, the synthesis of the N-linked oligosaccharides of *Schizosaccharomyces pombe* are not well understood. The initial step in the assembly of N-linked oligosaccharides seems to be similar to that of *S. cerevisiae* (3). However, the smallest N-linked oligosaccharides is $(Man)_9(GlcNAc)_2$, because *S. pombe* cells lack the ER Man_9-α1,2-mannosidase (4). Moreover, the N-linked oligosaccharides in *S. pombe* were elongated in the secretory pathway by the additions of 50-100 sugar residues consisting not only mannose but also galactose residues (5). We are very interested in the roles of galactose residues in the *S. pombe* oligosaccharides, because galactose residues are very common in the glycoproteins of higher eukaryotes. Recently some glycosylation defective mutants have been isolated from *S. pombe* (6-8), and this organism is certain to receive increased attention for studying glycosylation process.

We searched for mutants defective in oligosaccharide synthesis, especially that of galactosylation, to identify the role of galactomannan chains in *S. pombe.* We isolated *gms1* (for **g**alacto**m**annan **s**ynthesis defective) mutant that is deficient in glycosylation of cell surface glycoproteins by lectin-agglutination procedure, and galactose content in the *gms1* mutant polysaccharides was significantly reduced (9). In this communication, we describe the isolation of *gms1*$^+$ gene which is required for galactosylation of *S. pombe* cells. Gms1 protein has been found to share significant sequence similarity with mammalian sugar-nucleotide transporters. The Δ*gms1* cells lacked galactose residues in cell surface glycoproteins and were significantly decreased in UDP-galactose transport activity. Our data show that the lack of galactose residues in Δ*gms1* glycoproteins is caused by the defect of a transport of UDP-Gal into the lumen of Golgi apparatus.

MATERIALS AND METHODS

Strains, media and genetic methods. *E. coli* strain XL1-Blue (Stratagene, La Jolla, CA) was used for all cloning procedures. The wild-type *S. pombe* strains TP4-1D (*h+ leu1 his2 ura4 ade6-M216*) and TP4-5A (*h− leu1 ura4 ade6-M210,* 10) were obtained from Dr. T. Toda (ICRF). The *gms1-1* mutant (TP4-5A *gms1*) was isolated using a lectin-

[1] Correspondence to Kaoru Takegawa, Dept. of Bioresource Science, Fac. of Agriculture, Kagawa Univ., Miki-cho, Kagawa 761-07, Japan. Fax: +81-878-98-7295. E-mail: takegawa@ag.kagawa-u.ac.jp.

Abbreviations: UDP-Gal, UDP-galactose; UDP-GlcNAc, UDP-N-acetylglucosamine; CMP-SA, CMP-sialic acid; Con A, concanavalin A lectin; PNA, peanut (*Arachis hypogaea*) lectin; Gms1p, Gms1 protein; ER, endoplasmic reticulum.

0006-291X/97 $25.00

agglutination procedure as described (9). These strains were cultured in standard rich (YPD) and synthetic minimal (MM) media as described by Moreno *et al.* (11) at 28°C. Standard procedures for *S. pombe* manipulation were as described (12). *S. pombe* cells were transformed by the lithium acetate method as described (13).

Construction of plasmids. Conventional recombinant DNA methods were used in the construction and propagation of all plasmids (14). The *S. pombe* shuttle vector pAL-KS$^+$ (*LEU2,* multi-copy plasmid) was kindly provided by Dr. C. Shimoda (Osaka-City Univ.). Genomic library of *S. pombe,* pAL-SK$^+$ *Xba*I, which contained fragments of the genomic DNA of *S. pombe,* was kindly provided by Dr. K. Okazaki (Kazusa DNA Research Institute). pNT1 was constructed by inserting the subcloned 1.4-kb *Hinc*II-*Bgl*II genomic DNA fragment of *gms1*$^+$ gene into the *Sma*I-*Bam*HI site present in pAL-KS$^+$.

Disruption of the gms1$^+$ *gene.* Disruption of the *gms1*$^+$ gene was carried our as follows. The open reading frame of the *gms1*$^+$ gene was eliminated and the *S. pombe ura4*$^+$ cassette was inserted (15). The linearlized DNA fragment containing the disrupted *gms1*$^+$ gene was used to transform the haploid strain, TP4-1D to produce strain NTD-1. Replacement of the wild-type allele by the disrupted gene was confirmed by Southern analysis of several transformants (data not shown).

UDP-galactose transporting assay. Transport of UDP-Gal was directly examined with the P100 fraction consisting mainly Golgi and ER membranes obtained from *S. pombe* wild-type and *gms1*Δ cells as described (16). *S. pombe* cells were grown in MM medium, spheroplasted, and resuspended in 0.1M KCl, 15 mM Hepes (pH7.5), 3 mM EGTA and 10% glycerol. The cells were lysed by vortexing in the presence of glass beads and protease inhibitors. The lysates were cleared by centrifugation at 1,000 *g* for 10 min, and the resulting supernatant was centrifuged at 100,000 *g* for 1 hr at 4°C to generate P100 and S100 fractions. P100 fractions were incubated at 30°C in a reaction mixture which contained 250 mM sucrose, 1 mM $MgCl_2$, 150 mM KCl, 50 mM dimercaptoethanol, 8 μM UDP-galactose, and 1 μCi of UDP-[^{3}H]Gal. The reaction was initiated by the addition of membranes. The reaction mixture was diluted 10-fold with ice-cold reaction mixture without UDP-[^{3}H]Gal, and poured over a nitrocellulose filter. The filter was dried and the radioactivity trapped on the filter was determined by scintillation spectrometry. As a control for nonspecific adsorption, the reaction was stopped immediately after the start, and the radioactivity remaining on the nitrocellulose membrane filter under the conditions was subtracted as a background value.

Analytical methods. N-linked polysaccharides of cell surface glycoproteins from wild-type and Δ*gms1* mutant cells were prepared as described (9). Deuterium-exchanged polysaccharide alditol was measured on a JEOL JNM GX-400 spectrometer operated at 400 MHz in the Fourier transform mode at 27°C. The sugar compositions of *S. pombe* polysaccharides were analyzed by HPLC as described (9). Peroxidase-conjugated Con A and PNA lectins were used for the lectin-staining of *S. pombe* cells as described (9).

RESULTS

Isolation of a gms1$^+$ *gene from fission yeast.* Previously, we have described that the *gms1* mutant was extremely sensitive to vanadate, and could not grow on YPD plates containing 1 mM orthovanadate (9). This vanadate sensitivity was used to clone the wild-type gene affected by this mutation. The *gms1-1* cells were transformed with a pAL-SK$^+$ (*LEU2*) based *S. pombe* genomic library. Approximately 10,000 Leu$^+$ transformants were obtained, and four of which were found to be vanadate resisitant. The four complementing plasmids were amplified in *E. coli* and restriction enzyme mapped. Only 6-kb of sequence was common to all plasmid isolates. Further subcloning mapped the complementing activity to a 1.4-kb fragment (Fig. 1A). DNA sequence analysis of the 1.4-kb minimum complementing fragment revealed the presence of a single open reading frame predicted to code for a protein of 313 amino acids with three potential N-glycosylation sites (Fig. 1B). The amino acid sequence of Gms1p encodes a highly hydrophobic protein, and the N-terminal hydrophobic region that could serve as a signal sequence has not been found. The hydropathy profile and secondary structure prediction suggest that the Gms1p encodes an intrinsic membrane protein with between six and eight putative transmembrane domains (Fig. 1C).

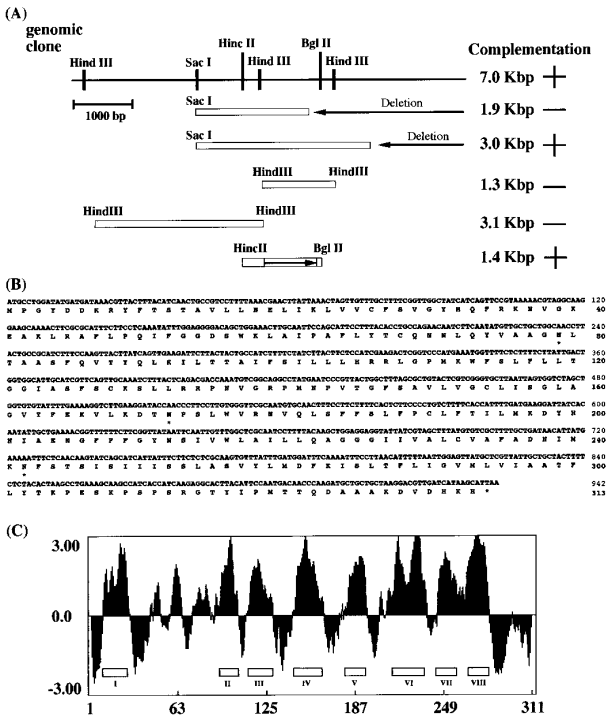

FIG. 1. Sequence analysis of the *gms1*$^+$ gene. (A) Restriction map of the isolated genomic 7-kb *Xba*I fragment, and complementation analysis of different subclones. If this fragment complements (+) or does not complement (−) of the *gms* phenotype of *gms1-1.* (B) Nucleotide and deduced amino acid sequence of *gms1*$^+$. The three potential N-glycosylation sites are indicated by asterisks. (C) Hydropathy plot of Gms1p. The plot was calculated with a window size of eight amino acids using the hydropathy values of Kyte and Doolittle (25). Boxes indicated the positions of putative membrane spanning segments.

A comparison of the deduced Gms1p amino acid sequence with sequence of other known proteins in the EMBL and GenBank data bases with the BLAST search revealed significant degrees of similarity with human UDP-Gal transporter (40% identity and 63% similarity) (17), murine CMP-sialic acid transporter (34%, 18), and hypothetical 29.4 kDa protein ZK370.7 on chromosome III of *Caenorhabditis elegans* (36% , 19)(Fig. 2). These results strongly suggest that the cloned gene encodes the UDP-Gal transporter of *S. pombe.*

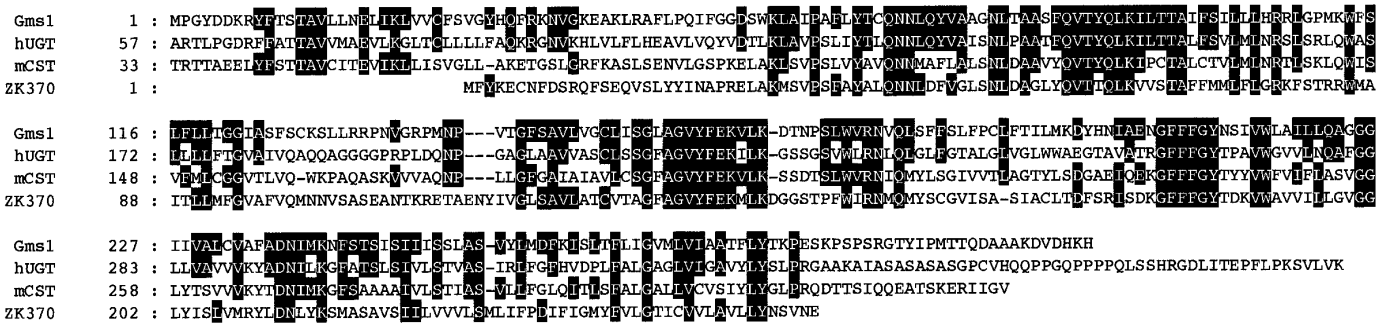

FIG. 2. The deduced amino acid sequence of Gms1 protein. Alignment of the amino acid sequence of Gms1 protein (Gms1) and human UDP-galactose transporter (hUGT), murine CMP-silalic acid transporter (mCST), and *C. elegans* ZK370.7 (ZK370).

Disruption of the gms1$^+$ gene. To ascertain the phenotypic consequence of the loss of *gms1$^+$* gene product function, one step gene replacement of *gms1$^+$* was carried out. We found that cells containing a disruption of the *gms1$^+$* gene were viable and showed almost identical phenotypes to those of the *gms1-1* mutant. The Δ*gms1* cells did not bind with the terminal galactose-specific lectin, PNA (Fig. 3A). Moreover, the Δ*gms1* cells bound with high affinity to Con A lectin, which is specific for mannose residues. Colonies of wild-type and Δ*gms1* cells grew at 26°C and 36°C; Δ*gms1* cells did not exhibit a temperature-sensitive growth defect (Fig. 3B). The Δ*gms1* strain showed strong sensitivity to vanadate and hygromycin B, and could not grown on YPD plates containing 1mM orthovanadate and 25 μg/ml hygromycin B (Fig. 3B). The introduction of plasmid pNT-1 into Δ*gms1* strain restored these phenotypes (Fig. 3A and B). The genomic DNA present within plasmid pNT-1 was therefore capable of complementing all Δ*gms1* strain phenotypes examined.

The Δgms1 strains lacked galactose residues in cell surface glycoproteins and decreased in UDP-Gal transport activity. The chemical structure of cell wall polysaccharides in Δ*gms1* strain was studied. Mannose and galactose are the component sugars at a ration of 1 : 1.2 in the wild-type polysaccharides. In contrast, the composition of sugars in the *gms1-1* mutant polysaccharides was Man : Gal = 1 : 0.18 (9). We have found that the polysaccharides of Δ*gms1* consisted exclusively of mannose residues, and galactose residues

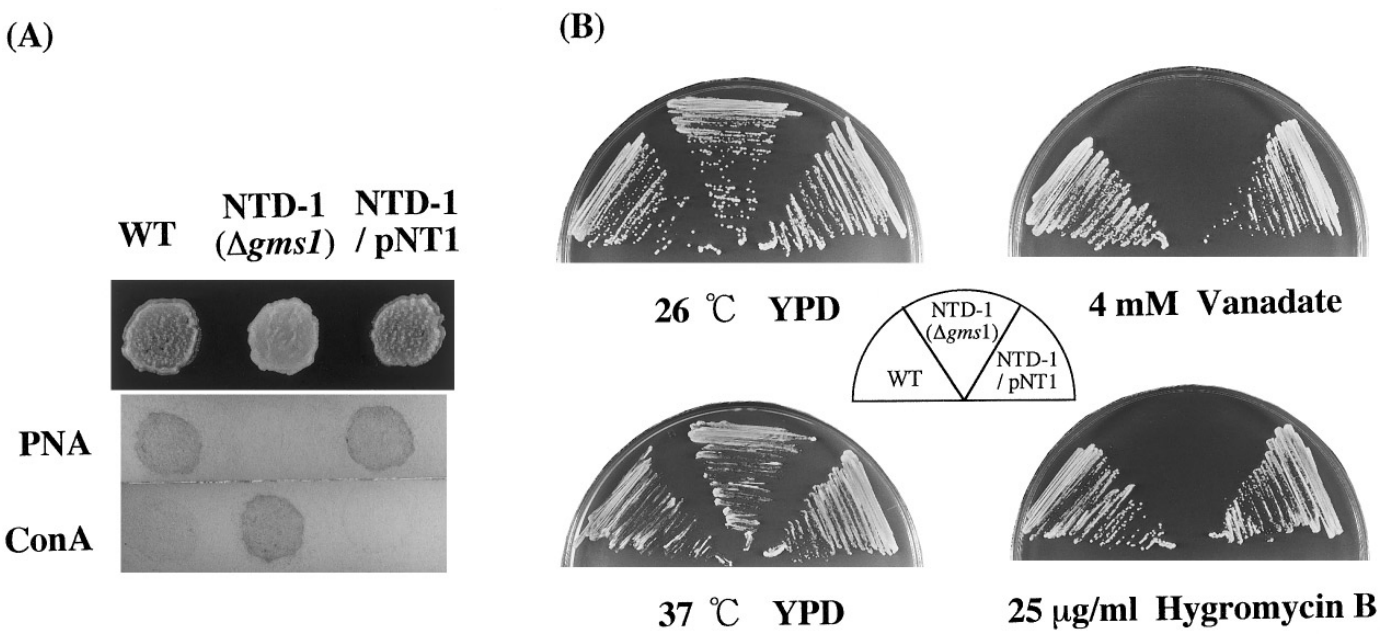

FIG. 3. Characterization of the glycosylation-defective and growth phenotype of NTD-1 (Δ*gms1*) strain. (A) The dot-spot staining assay of the patches showed the agglutination with PNA or Con A lectins (9). (B) The cells were streaked onto 26°C and 37°C YPD, 26°C YPD-4 mM vanadate, or 26°C YPD-25 μg/ml hygromycin B plates, and incubated for 3 to 6 days. WT, TP4-1D (wild-type); NTD-1, NTD-1 (Δ*gms1*); and NTD-1/pNT1, NTD-1 carrying plasmid pNT1.

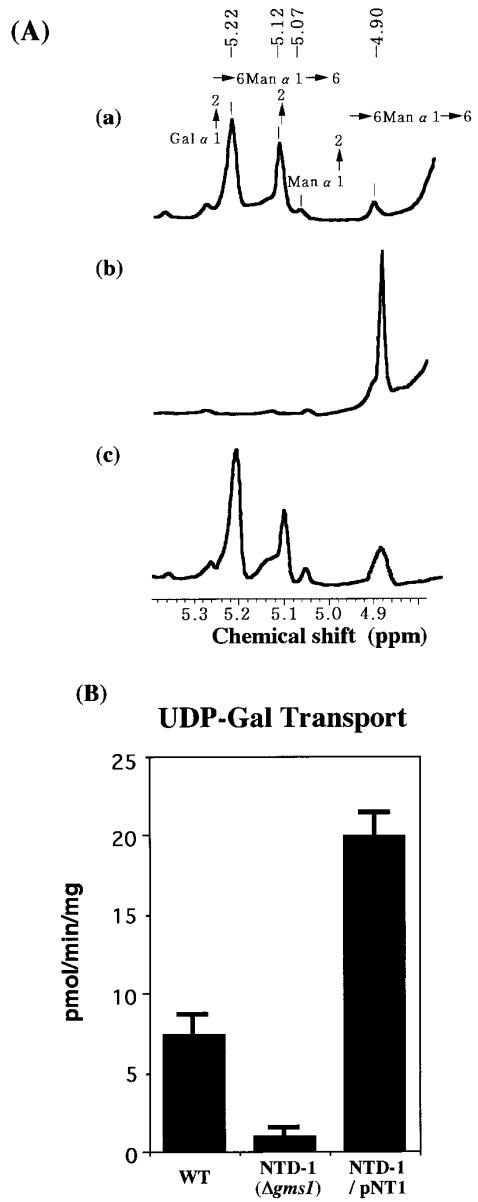

FIG. 4. Analysis of N-linked polysaccharide structure and UDP-Gal trasnport activity of Δ*gms1* strain. (A) Structural-reported group regions of 400 MHz ^{1}H-NMR spectra of TP4-1D (wild-type) (a), NTD-1 (Δ*gms1*) (b), and NTD-1 carrying plasmid pNT1 (c). (B) UDP-Gal transport activity of membrane vesicles from TP4-1D (WT), NTD-1 (Δ*gms1*), and NTD-1 carrying plasmid pNT1 (NTD-1/pNT1).

were completely lost by sugar-composition analysis. To confirm the absence of galactose residues in the cell wall glycoproteins of Δ*gms1* cells, the ^{1}H-NMR spectra of the wild-type and Δ*gms1* polysaccharides were compared (Fig. 4A). The NMR spectrum of Δ*gms1* polysaccharides was quite different from that of wild-type. The anomeric proton region the spectrum showed mostly a single signal at 4.91 ppm corresponding to 6-*O*-substituted α-mannopyranoside residues, and the signal at 5.22 ppm, mainly terminal galactose linked α1,2 as side chains (6,9), was completely dissapeared. These results showed that the α1,2-linked galactose resiudues was not attached in the glycoproteins of the Δ*gms1* strain.

Transport of UDP-Gal was directly examined with P100 fractions obtained from wild-type and Δ*gms1* cells. The membrane vesicles from Δ*gms1* cells significantly decreased the activity of transporting UDP-Gal (Fig. 4B). Moreover, upon introduction of *gms1*$^+$ gene (multi-copy plasmid) into Δ*gms1* cells, the membrane vesicles from the transformants recovered and increased the UDP-Gal transporting activity (Fig. 4B). These results showed that the Gms1p was very important for the incorporation of UDP-galactose into the lumen of Golgi in *S. pombe*.

DISCUSSION

Recent progress on the mammalian nucleotide-sugar transporter genes has provided us to identify the *gms1*$^+$ gene product of *S. pombe*. By analogy to *S. cerevisiae*, the outer N-linked polysaccharide chains of *S. pombe* probably occurs in the lumen of Golgi apparatus. UDP-Gal, the substrate for galactosyltransferase, must be transported from the cytosol, via Golgi membrane transporters into the lumen for the biosynthesis of the polysaccharide chains. We showed that the *gms1*$^+$ gene encodes an human UDP-Gal transporter homologue and Gms1p is essential for the incorporation of UDP-Gal into Golgi vesicles by *in vitro* transport assay. The *gms1*$^+$ gene is the second report of sugar-nucleotide transporter in yeasts. The mannan chains of *Klyveromyces lactis* mannoproteins have terminal α1,2-linked GlcNAc residues (20). An *mnn2-2* mutant of *K. lactis* that lacks terminal GlcNAc in its mannan chains, and shows to have a specific defect in transport of UDP-GlcNAc into the lumen of Golgi vesicles (21). UDP-GlcNAc transporter of *K. lactis* was cloned by complementation of the *mnn2-2* mutation, and consisted of 328 amino acids and had between five and eight transmembrane domains (22). We examined sequence comparison between Gms1p and *K. lactis* UDP-GlcNAc transporter, but the homology between these two proteins were very low. The UDP-GlcNAc transporter of *K. lactis* has a leucine zipper motif at the C-terminal region (22). Moreover, murine CMP-SA transporter has also leucine zipper motif at the N-terminal region (18), which is thought to be involved in protein oligomerization (23). In contrast, human UDP-Gal transporter and Gms1p do not have leucine zipper motif. Efforts are underway to characterize the structure-function relationship of Gms1p.

Galactose residues are not added to secreted glycoproteins or mannoproteins in *S. cerevisiae* in general (2). Recently Schwientek *et al.* (24) reported the expression of human β1,4-galactosyltransferase in *S. cerevisiae*, and expression of the galactosyltransferase-*MNT1* fusion was able to allow the galactosylation of a number

of secreted *S. cerevisiae* proteins. These results suggest that UDP-Gal is also present in the lumen of the Golgi by *S. cerevisiae* UDP-Gal transporter-like protein. However, we could not find UDP-Gal transporter-related genes from *S. cerevisiae* database by BLAST search. Therefore UDP-Gal may be incorporated into the lumen of Golgi by different mechanisms in *S. cerevisiae*. We are attempting the expression of Gms1p in *S. cerevisiae* cells.

Interestingly, although the Δ*gms1* strains do not contain any galactose residues in the cell wall glycoproteins, the Δ*gms1* strain is viable and can grow even at higher temperature at 36°C. These results suggest that the addition of galactose residues to cell surface glycoproteins may not be essential for growth of *S. pombe* cells. Further characterization of the Δ*gms1* strain will help us to better understand the biological roles of galactose residues of glycoproteins and cell wall galactomannoproteins in *S. pombe* cells.

ACKNOWLEDGMENTS

We gratefully acknowledge the gift from Takashi Toda, Chikashi Shimoda, Koei Okazaki of the *S. pombe* strains and plasmids. We thank Clinton Ballou, Scott Emr, Nobuhiro Ishida, and Masao Kawakita for valuable discussions and unpublished information. This work was supported in part by a Grant-in-aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (to K.T.). The *gms1*$^+$ nucleotide sequence will appear in DDBJ, EMBL, and GenBank nucleotide sequence databases with the following accession number D89616.

REFERENCES

1. Ballou, C. E. (1990) *Methods Enzymol.* **185,** 440–470.
2. Herscovics, A., and Orlean, P. (1993) *FASEB J.* **7,** 540–550.
3. Zou, J., Scocca, S., and Krag, S. S. (1995) *Arch. Biochem. Biophys.* **317,** 487–496.
4. Ziegler, F. D., Gemmill, T. R., and Trimble, R. B. (1994) *J. Biol. Chem.* **269,** 12527–12535.
5. Moreno, S., Ruiz, T., Sanchez, Y., Villanueva, J. R., and Rodrigez, L. (1985) *Arch. Microbiol.* **142,** 370–374.
6. Ballou, C. E., Ballou, L., and Ball, G. (1994) *Proc. Natl. Acad. Sci. USA* **92,** 2790–2794.
7. Ballou, L., and Ballou, C. E. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 2790–2794.
8. Huang, K. M., and Snider, M. D. (1995) *Mol. Biol. Cell* **6,** 485–496.
9. Takegawa, K., Tanaka, N., Tabuchi, M., and Iwahara, S. (1996) *Biosci. Biochem. Biotech.* **60,** 1156–1159.
10. Ohkura, H., Kinoshita, N., Miyatani, S., Toda, T., and Yanagida, M. (1989) *Cell* **57,** 997–1007.
11. Moreno, S., Klar, A., and Nurse, P. (1991) *Methods Enzymol.* **194,** 795–823.
12. Alfa, C., Fantes, P., Hyams, J., McLoed, M., and Warbrick, E. (1993) *in* Experiments with Fission Yeast: A Laboratory Course Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
13. Okazaki, K., Okazaki, K., Kume, K., Jinno, S., Tanaka, K., and Okayama, H. (1990) *Nucl. Acids. Res.* **18,** 6485–6489.
14. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *in* Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
15. Grimm, C., Kohli, J., Murray, J., and Maundrell, K. (1988) *Mol. Gen. Genet.* **215,** 81–86.
16. Waldman, B. C., and Rudnick, G. (1990) *Biochemistry* **29,** 44–52.
17. Miura, N., Ishida, N., Hoshino, M., Yamauchi, M., Hara, T., Ayusawa, D., and Kawakita, M. (1996) *J. Biochem.* **120,** 236–241.
18. Eckhardt, M., Muhlenhoff, M., Bethe, A., and Gerardy-Schahn, R. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 7572–7576.
19. Wilson, R. et al. (1994) *Nature* (London), **368,** 32–38.
20. Raschke, W. C., and Ballou, C. E. (1972) *Biochemistry* **11,** 3807–3812.
21. Abeijon, C., Mandon, E. C., Robbins, P. W., and Hirschberg, C. B. (1996) *J. Biol. Chem.* **271,** 8851–8856.
22. Abeijoin, C., Robbins, P., and Hirschberg, C. B. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 5963–5968.
23. Buckland, R., and Wild, F. (1989) *Nature* (London), **338,** 547.
24. Schwientek, T., Narimatsu, H., and Ernst, J. F. (1996) *J. Biol. Chem.* **271,** 3398–3405.
25. Kyte, J., and Doolittle, R. F. (1982) *J. Mol. Biol.* **157,** 105–132.