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A mutation that prevents glucosylation of the lipid-linked oligosaccharide precursor leads to underglycosylation of secreted yeast invertase

(*Saccharomyces cerevisiae*/endoglucosaminidase H/proton NMR/dolichol phosphoglucose/mannoprotein)

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ABSTRACT **A mutant of *Saccharomyces cerevisiae* with the genotype *mnn1 mnn2 mnn9 gls1* synthesizes mannoproteins with oligosaccharides having the composition $Glc_3Man_{10}GlcNAc_2$- owing to the *mnn9* defect, which prevents synthesis of the outer chain, the *mnn1* defect, which prevents branching of the core, and the *gls1* mutation, which prevents deglucosylation of the resultant glycoprotein as a consequence of a defective glucosidase-I [Tsai, P.-K., Ballou, L., Esmon, B., Schekman, R. & Ballou, C. E. (1984) *Proc. Natl. Acad. Sci. USA* 81, 6340–6343]. (The *mnn2* defect is not expressed in presence of the *mnn9* mutation.) This strain spontaneously forms new colonies in which *gls1* is suppressed owing to a defect in synthesis of dolichol phosphoglucose, the glucosylation substrate. The new mutant, designated *mnn1 mnn2 mnn9 gls1 dpg1*, synthesizes and secretes invertase (EC 3.2.1.26) that has a higher mobility on native gel electrophoresis than that made by the parent strain, the consequence of a reduction in both the size and the number of carbohydrate chains. The mannoprotein chains have the *mnn1 mnn9* structure ($Man_{10}GlcNAc_2$-), and the invertase is resolved by gel electrophoresis in sodium dodecyl sulfate into two major and two minor bands that represent homologs with about 4–7 carbohydrate units, in contrast to about 8–11 chains in the parent strain. Thus, the inability to glucosylate the lipid-linked precursor reduces the efficiency of glycosylation of the protein chains. The genetic defect is in synthesis of the glucose donor dolichol phosphoglucose, but the mutation is nonallelic with the reported *alg5-1* mutation, which has a similar phenotype [Runge, K. W., Huffaker, T. C. & Robbins, P. W. (1984) *J. Biol. Chem.* 259, 412–417].**

Several studies have suggested that the glucose units on the dolichol-linked oligosaccharide precursor facilitate the transfer of the carbohydrate part to asparagine in polypeptide chains (1–3). On the other hand, a mutant of *Saccharomyces cerevisiae* that is unable to remove the glucose units from the oligosaccharide chains on glycosylated proteins (4), owing to a defect (*gls1*) in glucosidase-I (5), appears to act normally in the subsequent processing steps.

We have taken advantage of the simplified carbohydrate chains made in the *mnn1 mnn2 mnn9* mutant of *S. cerevisiae* (6) to demonstrate the presence of the glucose units on invertase (EC 3.2.1.26) made in the *mnn1 mnn2 mnn9 gls1* strain (7). The secreted invertases from both of these strains migrate on a nondenaturing gel as relatively sharp bands, with that from the strain with the *gls1* mutation traveling slightly slower than that from the *mnn1 mnn2 mnn9* strain owing to the extra hexose units. We have now observed that spontaneous mutations occur in the *mnn1 mnn2 mnn9 gls1* strain that lead to colonies with a slightly altered morphology, the cells of which secrete invertase that has a higher electrophoretic mobility than the *mnn1 mnn2 mnn9* strain, even though the mannoproteins made in this new mutant have carbohydrate chains that lack the glucose units and are identical with those of the latter strain. This apparent suppression of the *gls1* mutation is due to the spontaneous loss in the ability to synthesize the glucose donor, dolichol phosphoglucose, not in a loss of the *gls1* defect. The increased mobility of the invertase is a consequence of a reduced level of *glycosylation*, which confirms that the failure to *glucosylate* the precursor reduces the efficiency of transfer of the oligosaccharide chains to polypeptides during their passage into the lumen of the endoplasmic reticulum (3, 8).

MATERIALS AND METHODS

Materials. All yeast strains were derived from *S. cerevisiae*. The *mnn1 mnn2 mnn9* (9) and *mnn1 mnn2 mnn9 gls1* (7) strains are mannoprotein mutants of *S. cerevisiae* X2180 and came from previous studies, whereas the *alg5-1* and *alg6-1* mutants were derived from *S. cerevisiae* DBY640 and were supplied by Runge *et al.* (3). A strain with auxotrophic markers *leu2 his4 ura3* (JRY438) was obtained from Jasper Rine of this department and was used to construct strains with the genotypes α *alg5-1 mnn9 leu2 his4* and a *dpg1 mnn9 ura3 his4*, which were mated on minimal plates containing histidine to give the desired diploid. Sources of all common reagents are given elsewhere (6, 7, 9). Dolichol monophosphate was from Sigma and UDP-[6-^{3}H]glucose (4.7 Ci/mmol; 1 Ci = 37 GBq) was from Amersham.

Methods. Yeasts were grown and induced for invertase production, and the invertases were analyzed by nondenaturing gel electrophoresis according to Tsai *et al.* (7). Invertases were purified and analyzed by sodium dodecyl sulfate gel electrophoresis as described by Lehle *et al.* (10). The internal (nonglycosylated) invertase of all strains was precipitated by 60% saturated ammonium sulfate, the 4AL external (glycosylated) invertase was precipitated by 80% saturated ammonium sulfate, while the external invertases of the *mnn1 mnn2 mnn9* and wild-type strains were soluble in 80% saturated ammonium sulfate, properties that reflect the different amounts of carbohydrate. The invertases were further purified by ion-exchange chromatography on DEAE-Sephacel and SP-Trisacryl by use of a 0–0.6 M NaCl gradient and by gel filtration on Sephacryl S-300. Digestions with endoglucosaminidase H were done according to Trimble and Maley (11). The isolation of bulk cell wall mannoprotein and the preparation of the asparagine-linked oligosaccharides and their characterization by proton nuclear magnetic resonance followed the procedures of Tsai *et al.* (6).

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Methods for the incorporation of labeled sugars into lipid-linked precursors and for their characterization have been published (3). Thin-layer chromatography of lipids was done on silica gel 60 precoated sheets (MC/B Manufacturing, no. 5538) with the solvent chloroform/methanol/water (60:25:4, vol/vol), and radioactivity was monitored by scintillation counting of portions of the gel scraped from the plate. Thin-section electron microscopy, on cells that were prefixed with tris(1-aziridinyl)phosphine oxide/acrolein, and genetic analysis of yeast strains were done according to Ballou *et al.* (9).

The presence of the *mnn1* mutation is scored by a failure of the cells to agglutinate with the anti-α1→3-mannosyl-specific serum and the *mnn2* mutation is scored by a positive reaction of the cells with anti-α1→6-mannosyl-specific serum. The *mnn9* mutation eliminates the outer chain and the reaction with the latter serum, and it can be scored by the fact that it leads to small colony size and a clumpy cell morphology. Thus, the *mnn2* defect, which affects only the outer chain, is not expressed in presence of *mnn9*. The invertases secreted by the *mnn1 mnn2 mnn9* and *mnn1 mnn2 mnn9 gls1* strains have slightly different rates of migration on nondenaturing gel electrophoresis, and they can be distinguished in this manner owing to the *mnn9* background, but the *mnn1 mnn2* and *mnn1 mnn2 gls1* invertases do not differ significantly in this regard. The *gls1* defect can also be detected by a glucosidase assay (5).

RESULTS

Spontaneous Mutants of the *mnn1 mnn2 mnn9 gls1* Strain Secrete Invertase with an Increased Electrophoretic Mobility. The *mnn1 mnn2 mnn9 gls1* strain grows slowly (7), and the cells show the clumpy morphology that is characteristic of the *mnn9* mutant (9). A streak of the former mutant strain, on a yeast extract/peptone/glucose/0.5 M KCl agar plate, after several weeks at 23°C, yielded numerous small colonies on the background that grew faster on the plate than the parent strain. Under a microscope, the cells of these colonies are noticeably larger and rounder than those of the parent strain, as if the walls were more plastic. The cell wall is readily digested by Zymolyase and is easily broken by stirring with glass beads, and thin-section electron microscopy reveals an unusual ease of stain penetration and an irregular septum and outer wall layer similar to that of the *mnn9* strain (data not shown) (9).

Nondenaturing gel electrophoresis of the invertases produced by the *mnn1 mnn2 mnn9* and *mnn1 mnn2 mnn9 gls1* strains and by an isolate of the new strain (initially called 4AL) are shown in Fig. 1. The invertases from the first two strains migrate as fairly sharp bands because all of the oligosaccharide chains have uniform structures, but the invertases differ in position owing to the extra 30 or so glucose units that are present in the strain carrying the *gls1* defect. The 4AL invertase migrates considerably faster than the *mnn1 mnn2 mnn9* invertase, and the diffuseness of the band suggests that it is heterogeneous. The bulk mannoprotein of the 4AL strain also migrates faster than that from the *mnn1 mnn2 mnn9* strain, which has been shown to have a M_r of 180,000 that is reduced to about 140,000 in cells incubated with tunicamycin (12). This suggests that the cell wall mannoprotein of the 4AL strain could also be deficient in carbohydrate chains.

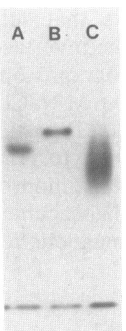

FIG. 1. Nondenaturing gel electrophoresis of the external invertase from various yeast strains. All samples contain some nonglycosylated internal invertase that migrates near the front. Lane A, *mnn1 mnn2 mnn9*; lane B, *mnn1 mnn2 mnn9 gls1*; lane C, 4AL. The bands were visualized by an activity stain (12).

The Enhanced Mobility of 4AL Invertase Results from a Reduction in the Size and Number of Carbohydrate Chains. A reduction in either the size or the number of the oligosaccharide chains on the polypeptide could lead to a heterogeneous invertase of increased mobility. To define the probable mechanism, bulk cell wall mannoprotein was isolated from the 4AL strain and digested with endoglucosaminidase H to release the asparagine-linked oligosaccharides, which were purified and analyzed by proton nuclear magnetic resonance (13). A single type of chain was obtained that had an anomeric proton spectrum identical to that of the *mnn1 mnn2 mnn9* oligosaccharide (Fig. 2) (6). Thus, the oligosaccharides have lost the 3 glucose units, but the fact that the invertase migrates faster than the *mnn1 mnn2 mnn9* enzyme indicates that the number of carbohydrate chains must also have been reduced.

To test this supposition, purified external invertases from the wild-type, *mnn1 mnn2 mnn9*, and 4AL strains were compared by gel electrophoresis in sodium dodecyl sulfate (Fig. 3). Clearly, the 4AL invertase (lane C) is smaller than the *mnn1 mnn2 mnn9* invertase (lane B) but, unexpectedly, both preparations show a heterogeneous pattern of major and minor bands, with the *mnn1 mnn2 mnn9* invertases ranging in molecular weight from 81,000 to 90,000, whereas the 4AL bands range from 71,000 to 80,000. These values are approximate owing to the high content of carbohydrate in the invertases, but if one assumes that the bands differ by single

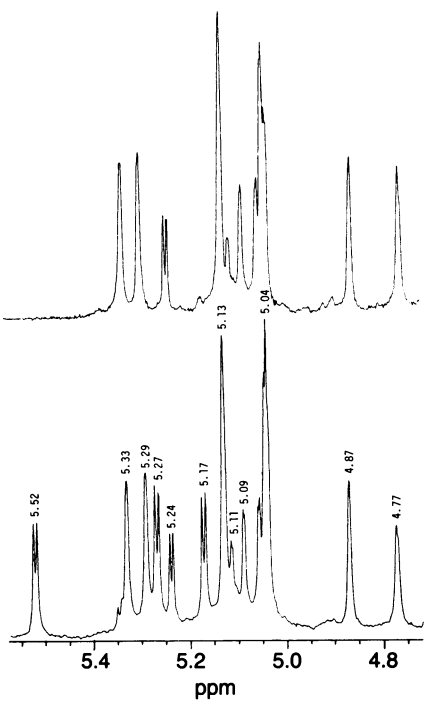

FIG. 2. Anomeric proton NMR spectra of mannoprotein oligosaccharides. The 4AL oligosaccharide (*Upper*) shows signals for 10 mannoses and 1 *N*-acetylglucosamine that have chemical shifts identical to those for the oligosaccharide from the *mnn1 mnn2 mnn9* strain (6), whereas the *mnn1 mnn2 mnn9 gls1* oligosaccharide (*Lower*) shows these same signals plus those for the 3 glucose residues at 5.17, 5.27, and 5.52 ppm (7).

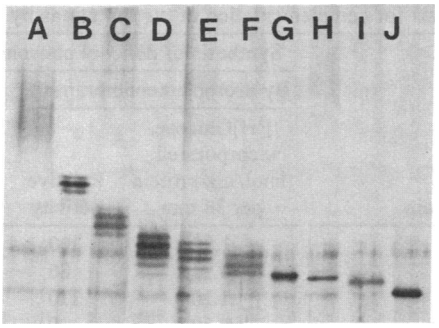

FIG. 3. Gel electrophoresis in sodium dodecyl sulfate of purified external invertases. Lanes A–C and J, intact invertases; lanes D–F, invertases treated with endoglucosaminidase under nondenaturing conditions; lanes G–I, invertases treated with endoglucosaminidase under denaturing conditions; lanes A, D, and G, wild-type invertase; lanes B, E, and H, *mnn1 mnn2 mnn9* invertase; lanes C, F, and I, 4AL invertase; lane J, internal invertase. The bands were visualized by a silver stain. The two diffuse bands near the bottom of all lanes are contaminants present in the reagents.

oligosaccharide chains, each chain appears to increase the M_r by 3000, although the actual mass of each chain is 2024. The internal invertase gives M_r 58,000 on this same gel, which is close to the true value of about 59,000. The difference between 71,000 and 58,000 divided by 3000 gives 4 as the number of carbohydrate chains on the fastest-migrating 4AL invertase band. Thus, this invertase preparation probably has homologs with 4 to 7 carbohydrate chains, whereas that from the *mnn1 mnn2 mnn9* strain would have homologs with 8 to 11 chains, the major band having 10 chains.

Under denaturing conditions, the invertases all are converted by endoglucosaminidase H digestion to single components, whose positions on the gel differ slightly, as expected from the different number of *N*-acetylglucosamine units left on asparagine. Digestion of the *mnn1 mnn2 mnn9* invertase preparation with endoglucosaminidase H under nondenaturing conditions (14) yields a new pattern of about six bands, which range from M_r 61,000 to M_r 73,000, with the fastest migrating band appearing to be completely deglycosylated. Similar treatment of the 4AL invertase gives a pattern of bands that migrate slightly faster. By counting the number of distinct nonoverlapping bands in these various preparations, we again conclude that the most highly glycosylated homolog in the *mnn1 mnn2 mnn9* invertase contains 11 carbohydrate chains, and that the 4AL invertase preparation contains homologs with 4 to 7 chains. Gel electrophoresis of the wild-type invertase (lane A) does not reveal bands because the carbohydrate component is so large and heterogeneous, but after endoglucosaminidase H digestion under nondenaturing conditions the pattern (lane D) is similar to that of the digested *mnn1 mnn2 mnn9* invertase except that the bands are slightly retarded due to the extra mannoses in the core that are absent from *mnn1* strains.

The New Mutation in Strain 4AL Affects the Synthesis of Dolichol Phosphoglucose. Genetic analysis of the 4AL strain revealed that it retained the *gls1* mutation. A cross with the *mnn1 mnn2* strain produced a diploid from which, following sporulation, a recombinant clone could be recovered with the *mnn1 mnn2 mnn9 gls1* phenotype (Table 1). Thus, the 4AL strain could be assigned the genotype *mnn1 mnn2 mnn9 gls1 sup1* (*sup1* designating a suppressor of *gls1*). The mechanism for suppression of the *gls1* defect that seemed most likely was a defect in the glucosylation pathway. Runge *et al.* (3) have already described such mutations that, when expressed in the *sec18* mutant, which accumulates invertase in the endoplasmic reticulum (8), show gel electrophoretic properties similar to those we find for invertase secreted by the 4AL strain.

Table 1. Genetic analysis of strain 4AL

Strains crossed	Recombinant tetrads obtained*
mnn1 mnn2 × 4AL†	*mnn1 mnn2 mnn9 gls1*
	mnn1 mnn2 mnn9 sup1
	mnn1 mnn2 gls1
	mnn1 mnn2 sup1
	mnn1 mnn2 mnn9 sup1
	mnn1 mnn2 mnn9 sup1
	mnn1 mnn2 gls1
	mnn1 mnn2 gls1
	mnn1 mnn2 mnn9
	mnn1 mnn2 gls1 sup1
	mnn1 mnn2 mnn9 gls1
	mnn1 mnn2 sup1
X2180 × 4AL	*mnn1 mnn2 mnn9 gls1*
	mnn1 mnn2 mnn9 gls1
	sup1
	sup1
sup1 × *mnn9*	*mnn9 sup1*
	mnn9
	sup1
	Wild type (X2180)

*Scoring of the tetrads is based on the phenotypes described in *Materials and Methods*.
†The 4AL strain was derived from the *mnn1 mnn2 mnn9 gls1* strain. *sup1* is the mutation that suppresses the *gls1* phenotype; after characterization, *sup1* was redesignated *dpg1*.

Analysis of the lipid-linked oligosaccharides that accumulated in the 4AL strain when incubated with [^{3}H]glucose showed that they had the size of $Man_8GlcNAc_2$-, whereas the wild-type strain accumulated this oligosaccharide along with the glucosylated form $Glc_3Man_8GlcNAc_2$- (data not shown). Further investigation revealed that membranes from the 4AL mutant were unable to synthesize dolichol phosphoglucose (Fig. 4, Table 2), and we concluded that the 4AL mutation might be allelic with the *alg5-1* mutation of Runge *et al.* (3). For genetic analysis, we constructed strains with the genotypes *α alg5-1 mnn9 leu2 his4* and *a sup1 mnn9 ura3 his4*, and we followed segregation of all markers in diploids obtained by mass mating. Although the diploids sporulated well, spore

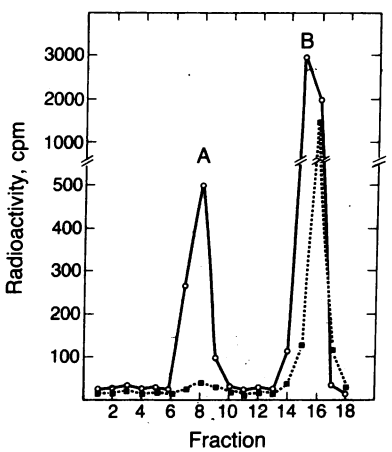

FIG. 4. Thin-layer chromatography of the lipid-soluble products from incubation of yeast membranes with UDP-[6-^{3}H]glucose. The solid line is for the *mnn1 mnn2 mnn9 gls1* strain and the dotted line is for the 4AL strain. The peaks for dolichol phosphoglucose (A) and steryl glucoside (B) are indicated. Peak A was characterized by the facts that its formation was dependent on added dolichol phosphate, that it bound to a DEAE-cellulose column and was eluted by 0.1 M ammonium formate, and that mild acid hydrolysis released a radioactive product with the chromatographic property of glucose (data not shown). Membranes were prepared from a Braun homogenate.

Table 2. Incorporation of radioactivity from UDP-[6-^{3}H]glucose into dolichol phosphoglucose and from dolichol phospho[6-^{3}H]glucose into lipid-linked core oligosaccharide by yeast membranes

Radioactive product	Radioactivity incorporated by membranes, cpm/mg protein			
	X2180	*mnn1 mnn2 mnn9*	*mnn1 mnn2 mnn9 gls1*	4AL
Neutral glucolipid*	15,000	14,000	8300	1820
Dolichol phosphoglucose†	1,750	1,400	1150	40
Lipid-linked core oligosaccharide‡	300	365	415	430

*Radioactivity in chloroform/methanol extract that was unbound to DEAE-cellulose, from an incubation of UDP-[6-^{3}H]glucose (160,000 cpm) with membranes (2 mg of protein) and exogenous dolichol phosphate. The neutral glucolipid is probably a steryl glucoside (15).

†Radioactivity in chloroform/methanol extract that bound to DEAE-cellulose and was eluted by 0.1 M ammonium formate. Thin-layer chromatography of this fraction showed a single radioactive component with the expected R_f for dolichol phosphoglucose (3).

‡From an incubation of purified dolichol phospho[6-^{3}H]glucose (40,000 cpm) with endogenous membrane acceptors. A control incubation without membranes gave 50 cpm. The labeled product was extracted from the membranes and treated with acid to hydrolyze the pyrophosphate linkage, and the released oligosaccharide was purified by gel filtration on a Bio-Gel P-4 column, from which it was eluted in a position for oligosaccharides with 12–15 hexose units.

viability was poor and most dissected asci gave only two or three clones. One ascus that gave four viable spores and one that gave three were examined for their invertase phenotypes. Lanes A and B of Fig. 5 represent the haploids that were mated and show the typical diffuse invertases of the *alg5-1* and 4AL strains, whereas lane C shows that a diploid formed in the mating makes invertase characteristic of the *mnn9* strain. The four lanes of D show invertases for a complete tetrad, all of which have the fast-migrating diffuseness of the mutant phenotype expected for a parental ditype, whereas the three lanes of E show the invertases of an incomplete tetrad in which recombination has occurred to give two clones with the *mnn9* phenotype expected of a nonparental ditype. Thus, the *alg5-1* and 4AL mutations are

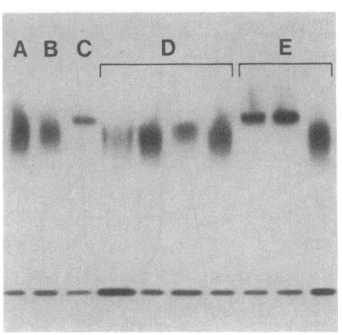

FIG. 5. Segregation of *alg5-1* and *dpg1* mutations determined by the phenotype of secreted invertases. Lane A, *a alg5-1 mnn9 leu2 his4* strain; lane B, *a dpg1 mnn9 ura3 his4* strain; lane C, diploid obtained by mating the two haploid strains; lanes D, invertases from the four clones of a complete tetrad representing a parental ditype; lanes E, invertases from three clones of an incomplete tetrad representing a nonparental ditype. The *alg5-1 mnn9* and *dpg1 mnn9* invertases give a diffuse fast-migrating invertase, whereas *mnn9* invertase gives a sharp somewhat slower-migrating band. The bands near the bottom of the gel are internal nonglycosylated invertases.

Table 3. Test for complementation of *alg* mutations by *dpg1*

Strain	Synthesis of dolichol phosphoglucose		
	By protoplast membranes*		By whole cells†
	[^{3}H]Glucose incorporated, pmol/mg protein per 30 min	Relative activity	Relative amounts
X2180	1.10	100	100
mnn9	0.94	86	ND
mnn9 alg6-1	1.20	110	ND
mnn9 alg5-1	0.10	9	ND
mnn9 dpg1	0.21	19	22
mnn9 alg5-1/mnn9 dpg1	1.08, 1.30‡	108	210

ND, not determined.

*The assays were performed in triplicate and the mixture contained UDP-[6-^{3}H]glucose, dolichol phosphate, and membranes from yeast protoplasts.

†A 5-ml culture (2×10^7 cells per ml) was incubated in 0.1% glucose with [2-^{3}H]glucose (50 μCi) for 1 hr at 30°C, after which the cells were harvested and the glucolipids were extracted and analyzed as in Fig. 4 and Table 2.

‡Results from two independently isolated zygotes.

nonallelic. Because this new mutation affects the synthesis of dolichol phosphoglucose, a glucolipid that is involved in more processes than just "asparagine-linked glycosylation" (3), we have designated it as *dpg1* and the original 4AL isolate as *mnn1 mnn2 mnn9 gls1 dpg1*.

The evidence that the *alg5-1* and *dpg1* mutations are nonallelic was supported by assays for dolichol phosphoglucose synthetase in membrane preparations and for the accumulation of the glucolipid in whole cells. Although the *alg5-1* strain showed little synthetase activity and the *dpg1* strain had about 20% of the wild-type activity, the heterozygous diploid containing *alg5-1* and *dpg1* had about 100% of the wild-type activity in protoplast membranes. Thus, the *dpg1* gene product is able to complement *alg5-1*. Further evidence for complementation came from assays for the cellular levels of dolichol phosphoglucose (Table 3). The heterozygous diploid contained more of this glucolipid than did the X2180 wild-type strain, whereas the *dpg1 mnn9* strain contained much less. We conclude that the low level of dolichol phosphoglucose synthesis in the *dpg1* strain is insufficient for glucosylation of the lipid-linked oligosaccharide precursor.

DISCUSSION

Our results demonstrate that the inability of *S. cerevisiae* cells to glucosylate the lipid-linked oligosaccharide precursor leads to underglycosylation of the invertase they make and secrete, in agreement with earlier conclusions (2, 3) that the glucose units have a role in facilitating the transfer of oligosaccharide to asparagine during glycoprotein synthesis. This effect is readily demonstrated in the *mnn1 mnn2 mnn9* mutant because this strain makes mannoproteins with small uniformly sized oligosaccharides (6) that are easy to analyze by gel electrophoresis (7). Precisely how the glucose units might facilitate transfer is unknown, although kinetic studies have shown that the effect is related to differences in V_{max} rather than K_m for the donor with and without the glucoses (2), whereas the presence of glucose units in the donor is reported to lower the K_m for a polypeptide acceptor (16). This suggests that the glucose units may affect catalysis by contributing to the enzyme–substrate binding energy much as do the several *N*-acetylglucosaminyl units that bind to subsites in lysozyme (17). We have presented evidence (7) that the glucotriose unit may have a hydrogen-bonded struc-

ture that gives it a well-defined conformation that could serve as a site for recognition by a protein involved in the glycosylation process, but the fact that the unit is 6 hexoses removed from the reaction site implies something novel about the interaction.

A notable property of the invertase made in the *mnn1 mnn2 mnn9 dpg1* mutant is that it consists of a limited mixture of homologs with about 4–7 oligosaccharide chains. After endoglucosaminidase H digestion under nondenaturing conditions, this invertase is converted to another set of four homologs that probably have from 0 to 3 oligosaccharide chains. From studies of Trimble *et al.* (18), we interpret these results to suggest that, as the nascent invertase polypeptide is fed into the lumen of the endoplasmic reticulum, a limiting rate of oligosaccharide transfer allows some of the polypeptide chains to fold up with less than a full complement of asparagine-linked carbohydrate. Subsequently, the glycosylation sites that are exposed on the surface of the invertase molecule can be more evenly substituted because the time during which they are accessible for reaction may not be limited by the kinetics of the folding process. It is probable, however, that even in wild-type invertase some underglycosylation of the endoglucosaminidase H-inaccessible sites exists, because it has been noted (10, 11, 14) that enzyme digestion of the invertase under nondenaturing conditions yields a pattern of incompletely deglycosylated electrophoretically separable bands. Here we show that the *mnn1 mnn2 mnn9* invertase preparation also gives several bands on gel electrophoresis in sodium dodecyl sulfate, and the major band has fewer than the possible maximum of 13 carbohydrate chains (19). These results indicate that our original supposition that the diffuseness of the 4AL invertase on a native gel reflected heterogeneity of glycosylation must not be correct because both the 4AL and *mnn1 mnn2 mnn9* invertases appear equally heterogeneous after gel electrophoresis in sodium dodecyl sulfate. The nature of underglycosylation of the 4AL invertase may, however, lead to a destabilization of the dimeric form of the enzyme as it migrates on the native gel, thus giving a diffuse band. Previous evidence for heterogeneity in glycosylation of invertase is also apparent in the electrophoretic pattern of radiolabeled material from the *sec18* mutant (4) in which the molecules accumulate with $Man_8GlcNAc_2$- chains and show a set of 3 to 4 bands.

The underglycosylation of mannoproteins in the *mnn1 mnn2 mnn9 gls1 dpg1* mutant has dramatic consequences for cell wall organization. Although the *mnn9* defect alone alters the wall integrity (9), owing apparently to a reduction in the length of some of the carbohydrate chains, the further decrease resulting from underglycosylation has consequences reminiscent of glucosamine auxotrophy (20) or Polyoxin D inhibition (21), which interfere with both chitin and glycoprotein synthesis. The major mannoprotein component of the cell wall has a high content of both asparagine- and hydroxyamino acid-linked carbohydrate, but interference with *N*-glycosylation alone significantly reduces the molecular size of this material (12). Thus, underglycosylation resulting from unavailability of glucosamine, from inhibition of oligosaccharide precursor synthesis or glucosylation, or from inhibition of oligosaccharide transfer to protein all have similar consequences and confirm that the mannose component of the wall mannoproteins has an important role in cell wall organization (22).

The frequency of spontaneous mutations that suppress the glucosidase-I defect is not high, but the fact that the mutants grow faster under certain conditions than the parent strains suggests that they may possess some selective advantage. Thus, although the inability to glucosylate the lipid-linked oligosaccharide precursor reduces the efficiency of protein glycosylation, the absence of the glucose units in the resulting glycoproteins may be advantageous at some later stage in their processing or function. If this is so, it could provide a rationale for the development in some organisms of a glycosylation pathway that involves a nonglucosylated precursor (23).

Lehle (15) and Runge *et al.* (3) have noted the synthesis of a neutral glucolipid, probably a steryl glucoside, along with dolichol phosphoglucose in incubations of yeast membranes with UDP-glucose, and we have made the same observation. Although Lehle (15) found that the relative amount of this neutral glucolipid is reduced when dolichol phosphate is added along with UDP-glucose in an incubation with membranes, suggesting that the latter is the true donor in the synthesis of this lipid, our finding that the amount of the neutral lipid synthesized from UDP-glucose by membranes from the *dpg1* strain is dramatically reduced suggests otherwise. Dolichol phosphoglucose is also reported to be involved in synthesis of ceramides in microsomes from BHK-21 cells (24), so it may have a more general role as a glucose donor in yeast than presently suspected because glycosides of both sterols and ceramide have been reported in *S. cerevisiae* (25).

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