Yeast mutants deficient in protein glycosylation

(asparagine-linked oligosaccharides/dolichol-linked oligosaccharides/endoplasmic reticulum enzymes)

TIM C. HUFFAKER* AND PHILLIPS W. ROBBINS†

Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

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ABSTRACT The synthesis of asparagine-linked oligosaccharides involves the formation of a lipid-linked precursor oligosaccharide that has the composition $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$. We have used a [^3H]mannose suicide selection to obtain mutants in yeast that are blocked in the synthesis of this precursor oligosaccharide. The *alg1* mutant accumulated lipid-linked GlcNAc_2 , *alg2* mutants accumulated $\text{Man}_{1-2}\text{GlcNAc}_2$, *alg3* mutants accumulated $\text{Man}_5\text{GlcNAc}_2$, *alg4* mutants accumulated $\text{Man}_{1-8}\text{GlcNAc}_2$, and *alg5* and *alg6* mutants accumulated $\text{Man}_9\text{GlcNAc}_2$. Some of these mutants appeared to transfer oligosaccharides other than $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$ from the lipid carrier to invertase. These aberrant protein-linked oligosaccharides were processed by the addition of outer chain residues in the *alg3*, *alg5*, and *alg6* mutants. There was virtually no outer chain addition in the *alg2* and *alg4* mutants. *alg4* was the only mutant that failed to secrete invertase.

The synthesis of asparagine-linked oligosaccharides of eukaryotic glycoproteins involves the formation of a lipid-linked precursor oligosaccharide that has the composition $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$. This precursor oligosaccharide is transferred as a unit from the lipid carrier (dolichol) to asparagine residues in the polypeptide chain. Subsequent processing of these protein-linked oligosaccharides yields the diverse array of structures found in eukaryotic cells (reviewed in ref. 1). In yeast, this processing is initiated with the removal of the glucose residues and a single specific mannose residue to produce protein-linked $\text{Man}_8\text{GlcNAc}_2$ (2). The addition of outer chain mannose residues to this core structure generates mature oligosaccharides ranging in size from the 9 mannose residue chains of carboxypeptidase Y (3) to the oligosaccharide chains of invertase that contain >50 mannose residues (4). Assembly of the precursor oligosaccharide on the lipid carrier has been proposed to occur by an ordered step-by-step addition of monosaccharide residues donated from either dolichol-linked or nucleotide sugars (5, 6). We have used a [^3H]mannose suicide selection (7) to obtain mutants in yeast that are deficient in asparagine-linked glycosylation (*alg* mutants). In this report we describe mutations in six complementation groups that appear to block various stages in the assembly of the precursor oligosaccharide.

EXPERIMENTAL PROCEDURES

Materials. *Saccharomyces cerevisiae* haploid strains DBY640 (MATa *ade2-101*), DBY741 (MATa *his4-539*), and DBY1033 (MATa *ura3-52*) were obtained from D. Botstein (Department of Biology, Massachusetts Institute of Technology). HMSF176 (MATa *sec18-1*) was obtained from P. Novick (Department of Biology, Massachusetts Institute of Technology). The CGS65 plasmid was obtained from D. Botstein. Antiserum specific for the protein portion of invertase was obtained from I. Schauer

(Department of Biochemistry, University of California, Berkeley).

Genetic Procedures. All mutants were derived from DBY640 and backcrossed to DBY741, DBY1033, and HMSF176. Conditions for crosses and sporulation were standard (8). Complementation analysis between two temperature-sensitive mutants was done by assaying the appropriate diploids for growth at 36°C. Otherwise, diploids were assayed for their ability to synthesize the lipid-linked precursor oligosaccharide at 36°C (7).

Immunoprecipitation of Invertase. Cells were transformed with CGS65 by the lithium acetate procedure (9). CGS65 is a 2- μm plasmid carrying *SUC2*, the structural gene for invertase (10). Transformants were grown to middle exponential phase at 26°C in minimal medium (0.67% Bacto-yeast nitrogen base/20 mg of adenine sulfate and histidine hydrochloride per liter) containing 5% glucose. A 3-ml aliquot was centrifuged. The cells were resuspended in 3 ml of medium containing 0.1% glucose and incubated at 26°C or 36°C for 30 min. [^{35}S]Methionine (500 μCi ; 1 Ci = 3.7×10^{10} Bq; New England Nuclear) was added and the cells were labeled at the same temperature for 30 min. After the addition of 3 ml of 20 mM sodium azide, the cells were centrifuged, washed with 10 mM sodium azide, and resuspended in 0.4 ml of spheroplasting solution (1 M sorbitol/20 mM potassium phosphate, pH 7.5/10 mM sodium azide/0.1% 2-mercaptoethanol/1 mg of zymolyase 60,000 per ml from Miles). After 20 min at 36°C, 0.4 ml of lysing solution (20 mM potassium phosphate, pH 7.5/0.2 M NaCl/2% Triton X-100/2 mM phenylmethylsulfonyl fluoride) was added. The lysate was centrifuged for 10 min at $15,000 \times g$; the supernatant was centrifuged for 60 min at $100,000 \times g$. The final soluble material was mixed with 5 μl of anti-invertase serum and incubated at 4°C for 16 hr.

A 40- μl aliquot of 20% (vol/vol) protein A-Sepharose CL-4B (Pharmacia) in PAS solution (10 mM potassium phosphate, pH 7.5/100 mM NaCl/0.5% Triton X-100/1 mg of bovine serum albumin per ml) was added. The mixture was incubated at 0°C for 30 min and centrifuged at $15,000 \times g$ for 1 min, and the supernatant was decanted. The beads were washed three times with PAS solution, twice with 10 mM Tris-HCl, pH 6.8/1% NaDodSO₄/2% 2-mercaptoethanol, and resuspended in 50 μl of the latter solution. The beads were boiled at 100°C for 3 min and centrifuged. The supernatant was mixed with an equal volume of 50 mM sodium citrate, pH 5.5/10 mM sodium azide. For endo-N-acetylglucosaminidase H (endo H) digestion, 3 μg of the enzyme per ml (25 units/mg) was added. After incubation at 37°C for 16 hr, an equal volume of 2 $\times$ concentrated sample buffer (0.1 M Tris-HCl, pH 6.8/2% NaDodSO₄/20%

Abbreviations: endo H, endo-N-acetylglucosaminidase H; Dol-P, dolichol phosphate.

* Present address: Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

† To whom reprint requests should be addressed.

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glycerol/10% 2-mercaptoethanol/0.01% bromphenol blue) was added. Samples were boiled at 100°C for 2 min and subjected to electrophoresis on 8% NaDodSO₄/polyacrylamide gels. The gels were treated with EN³HANCE (New England Nuclear), dried, and autoradiographed with Kodak XAR-5 film.

RESULTS

Lipid-Linked Oligosaccharide Synthesis in *alg* Mutants. The [³H]mannose suicide selection and screens described previously (7) were used to isolate mutants in yeast deficient in asparagine-linked glycosylation. Because it seemed likely that some mutations that interfered with protein glycosylation would be lethal, we used conditions that allowed the isolation of temperature-sensitive mutants. However, *alg* mutants were not required to be temperature sensitive for growth so that mutations could also be obtained in steps that were not essential for cell viability. Eighteen mutants have been obtained that fail to synthesize the lipid-linked precursor oligosaccharide (see below), and these fall into six complementation groups. There is one isolate each of *alg1*, *alg5*, and *alg6*, 3 isolates of *alg2*, 2 isolates of *alg3*, and 10 isolates of *alg4*. All alleles of *alg1*, *alg2*, and *alg4* are temperature sensitive for growth; they grow at 26°C but fail to grow at 36°C. All alleles of *alg3*, *alg5*, and *alg6* grow at both 26°C and 36°C.

When wild-type cells were labeled with [³H]mannose at 36°C, the major lipid-linked oligosaccharide observed was the precursor oligosaccharide, Glc₃Man₉GlcNAc₂ (Fig. 1F). Wild-type

cells also accumulated smaller amounts of Man₈GlcNAc₂, Man₇GlcNAc₂, and Man₅GlcNAc₂. In contrast, the *alg* mutants failed to synthesize the precursor oligosaccharide and accumulated intermediates, indicating that they were blocked at various stages in the assembly of lipid-linked Glc₃Man₉GlcNAc₂. The *alg1-1* mutant did not synthesize any mannose-labeled oligosaccharides and has been shown previously to be blocked in the addition of the first mannose residue (7). It accumulated GlcNAc₂. Each of the three alleles of *alg2* accumulated predominantly Man₂GlcNAc₂ and Man₁GlcNAc₂ (Fig. 1A, *alg2-1*). The two alleles of *alg3* accumulated Man₅GlcNAc₂ (Fig. 1B, *alg3-1*), and both *alg5-1* (Fig. 1D) and *alg6-1* (Fig. 1E) accumulated Man₉GlcNAc₂. All of the alleles of *alg4* accumulated multiple oligosaccharides ranging from Man₁GlcNAc₂ to Man₈GlcNAc₂ (Fig. 1C, *alg4-9*).

All of the *alg* mutations were recessive in heterozygous diploids. Analysis of each complementation group was conducted with a representative allele. Each group showed 2:2 segregation of the block in precursor oligosaccharide synthesis, indicating that this phenotype was due to a mutation in a single gene. The temperature-sensitive phenotypes of *alg1*, *alg2*, and *alg4* always segregated with the glycosylation defect.

Glycosylation of Invertase in *alg* Mutants Containing the *sec18-1* Mutation. The inability of the *alg* mutants to synthesize the precursor oligosaccharide suggested that they may transfer oligosaccharides smaller than Glc₃Man₉GlcNAc₂ to protein. The oligosaccharides transferred to invertase were examined in double mutants containing the *sec18-1* mutation and one of the *alg* mutations. The *sec18-1* mutation blocks secretion of proteins in yeast by blocking their transport from the endoplasmic reticulum (11). Because the addition of outer chain residues occurs after glycoproteins leave the endoplasmic reticulum (12), oligosaccharide processing is limited to the removal of the glucose residues and the mannose residue. With the exception of this limited processing, oligosaccharides found on invertase in cells carrying the *sec18-1* mutation will be those oligosaccharides that were transferred from lipid to protein. Invertase was immunoprecipitated from [³⁵S]methionine-labeled cells and examined by NaDodSO₄/polyacrylamide gel electrophoresis before and after endo H treatment. Endo H cleaves the di-*N*-acetylchitobiose unit that connects the oligosaccharide to the polypeptide chain (4). Of the oligosaccharide structures generated in the assembly of the precursor oligosaccharide, only those larger than Man₅GlcNAc₂ will be cleaved by endo H (13). Therefore, if the mutants transfer Man₅GlcNAc₂ or smaller oligosaccharides to protein, the carbohydrate chains of invertase should be resistant to endo H.

At 36°C, cells carrying *sec18-1* in an otherwise wild-type background made invertase that migrated in discrete bands with apparent *M_s* of 80,000–86,000 (Fig. 2, lane a). The bands presumably reflect differences in the number of core oligosaccharide chains attached to invertase. The nonglycosylated cytoplasmic form of invertase was present as a band at *M_r* 59,000. Endo H digestion produced a polypeptide containing ≈10 *N*-acetylglucosamine residues (4, 14) that migrated at an apparent *M_r* of 61,000 (Fig. 2, lane b). Invertase from *alg3-1 sec18-1*, *alg5-1 sec18-1*, and *alg6-1 sec18-1* cells also migrated in discrete bands (Fig. 2, lanes g, k, and m). These had higher mobilities than *sec18-1* invertase, indicating that the protein made in these cells contained less carbohydrate. Oligosaccharides on *alg5-1 sec18-1* and *alg6-1 sec18-1* invertase were sensitive to endo H (Fig. 2, lanes l and n). These endo H-treated proteins migrated slightly faster than *sec18-1* endo H-treated invertase, suggesting that they contained fewer *N*-acetylglucosamine residues. Most of the oligosaccharides on *alg3-1 sec18-1* invertase were resistant to endo H, but the shift in mobility indicated that some

FIG. 1. Lipid-linked oligosaccharides synthesized by the *alg* mutants at 36°C. Cells were grown at 26°C and shifted to 36°C for 30 min. [³H]Mannose was then added and the cells were labeled at 36°C for 10 min. The lipid-linked oligosaccharides were extracted and analyzed by gel filtration on Bio-Gel P-4. Details of this procedure have been described (7). G, Glc; M, Man. All oligosaccharides contain two GlcNAc residues.

FIG. 2. Invertase synthesized in *alg sec18-1* double mutants at 36°C. Invertase was immunoprecipitated from cells labeled at 36°C with [³⁵S]methionine, incubated with (+) or without (–) endo H, and analyzed by NaDodSO₄/polyacrylamide gel electrophoresis. WT is *sec18-1* in a wild-type background; all other strains contain *sec18-1* and the indicated *alg* mutation. Arrow indicates the position of cytoplasmic invertase.

endo H-sensitive oligosaccharides were present (Fig. 2, lane h). Invertase from *alg1-1 sec18-1*, *alg2-1 sec18-1*, and *alg4-9 sec18-1* cells migrated diffusely. Some of the *alg1-1 sec18-1* invertase appeared to be fully glycosylated but most contained less carbohydrate than *sec18-1* invertase (Fig. 2, lane c). *alg2-1 sec18-1* and *alg4-9 sec18-1* invertase were the most deficient in carbohydrate (Fig. 2, lanes e and i). *alg1-1 sec18-1* invertase appeared to be completely sensitive to endo H, and *alg2-1 sec18-1* invertase was partially sensitive to endo H, and *alg4-9 sec18-1* invertase was almost completely resistant to endo H (Fig. 2, lanes d, f, and j). The material in *alg4-9 sec18-1* cells that migrated faster than nonglycosylated invertase was probably produced by proteolytic cleavage of invertase at a sensitive site.

Glycosylation of Invertase in *alg* Mutants. The results obtained with *alg sec18-1* double mutants indicated that oligosaccharides other than Glc₃Man₉GlcNAc₂ were transferred to invertase at 36°C. The manner in which these aberrant core oligosaccharides are processed was examined in each of the *alg* mutants without the *sec18-1* block. In wild-type cells the addition of outer chain mannose residues produced invertase that migrated diffusely with an apparent *M_r* of 100,000–140,000 (Fig. 3, lane a). These elongated chains remained sensitive to endo H digestion (Fig. 3, lane b). *alg3-1*, *alg5-1*, and *alg6-1* cells also synthesized high molecular weight invertase, indicating that their glycoproteins contained outer chain residues (Fig. 3, lanes g, k, and m). However, in each case, these cells also produced invertase of higher mobility than wild-type invertase. All of the oligosaccharide chains on *alg5-1* and *alg6-1* invertase remained sensitive to endo H after elongation (Fig. 3, lanes l and n). Most of the oligosaccharides on *alg3-1* invertase were resistant to endo H, as was the case for the core oligosaccharides on *alg3-1 sec18-*

FIG. 3. Invertase synthesized in *alg* mutants at 36°C. Invertase was immunoprecipitated from cells labeled at 36°C with [³⁵S]methionine, incubated with (+) or without (–) endo H, and analyzed by NaDodSO₄/polyacrylamide gel electrophoresis. WT is wild type; all other strains contain the indicated *alg* mutation. Arrow indicates the position of cytoplasmic invertase.

1 invertase (Fig. 3, lane h). *alg1-1* invertase migrated diffusely with apparent *M_r*s of 62,000–140,000; the most prominent species were in the lower *M_r* range of 62,000–84,000 (Fig. 3, lane c). All of the oligosaccharides on *alg1-1* invertase appeared to be sensitive to endo H (Fig. 3, lane d). Only a very small fraction of *alg2-1* invertase migrated in the high *M_r* range (Fig. 3, lane e). Most of the oligosaccharides in *alg2-1* invertase were not elongated and remained endo H resistant (Fig. 3, lane f). *alg4-9* invertase was virtually identical to *alg4-9 sec18-1* invertase, indicating that no outer chain addition occurs in this mutant (Fig. 3, lanes i and j).

In the *alg3-1*, *alg5-1*, and *alg6-1* mutants, which are not temperature sensitive for growth, invertase made at 26°C was indistinguishable from that made at 36°C (Fig. 4, lanes g, h, and k–n). However, invertase synthesized in the temperature-sensitive *alg* mutants was more fully glycosylated at 26°C than at 36°C. *alg1-1* invertase appeared to be identical to wild-type invertase (Fig. 4, lanes a–d). *alg4-9* invertase was also elongated, but on average it contained slightly less carbohydrate than wild type (Fig. 4, lane i). In contrast to the results obtained at 36°C, *alg4-9* invertase made at 26°C was completely sensitive to endo H (Fig. 4, lane j). About half of the oligosaccharides on *alg2-1* invertase were elongated and some of the unelongated chains remained resistant to endo H (Fig. 4, lanes e and f).

Secretion of Invertase in *alg* Mutants. The *alg* mutants were examined for their ability to secrete invertase at 36°C. Secreted proteins were released into the supernatant by treating cells with an enzyme that removed the cell wall. Nonsecreted proteins remained inside the spheroplasts. These fractions were separated and analyzed for their invertase content. In wild-type cells all of the high molecular weight invertase was secreted.

FIG. 4. Invertase synthesized in *alg* mutants at 26°C. Invertase was immunoprecipitated from cells labeled at 26°C with [³⁵S]methionine, incubated with (+) or without (–) endo H, and analyzed by NaDodSO₄/polyacrylamide gel electrophoresis. WT is wild type; all other strains contain the indicated *alg* mutation. Arrow indicates the position of cytoplasmic invertase.

Some invertase that contained only core glycosylation and the cytoplasmic form of invertase remained in the spheroplast pellet (Fig. 5, lanes a and b). All of the invertase made in *sec18-1* cells was found in the spheroplast pellet (Fig. 5, lanes o and p). In *alg1-1*, *alg2-1*, *alg3-1*, *alg5-1*, and *alg6-1* cells all of the glycosylated invertase was in the spheroplast supernatant, with the exception of some molecules that contained only core glycosylation (Fig. 5, lanes c–h and k–n). Evidently, *alg2-1* spheroplasts lysed more easily than the other *alg* spheroplasts, because only about 10% of the cytoplasmic invertase was found in the spheroplast pellet. However, no glycosylated invertase was seen in the pellet fraction, even though it was made in far greater amounts. Therefore, we conclude that *alg2-1* invertase is secreted. In *alg4-9* cells most of the invertase was found in the pellet fraction (Fig. 5, lanes i and j). *alg4-9* is thus the only *alg* mutation that inhibits invertase secretion.

DISCUSSION

The *alg1-1* mutant accumulated GlcNAc₂-lipid at 36°C (7). Invertase made in the *alg1-1 sec18-1* double mutant ran as a smear, indicating heterogeneity in both the size of the protein-linked oligosaccharides and the number of sites glycosylated. These oligosaccharides appeared to be sensitive to endo H digestion, suggesting that *alg1-1* cells synthesized and transferred to protein oligosaccharides larger than Man₅GlcNAc₂. Because the *alg1-1* mutation affects the addition of the first mannose residues, it must be somewhat leaky at 36°C. Some of the heterogeneity may be due to transfer of GlcNAc₂ to protein. Although GlcNAc₂ would not be cleaved by endo H, the presence of GlcNAc₂ chains on invertase may not give a detectable de-

FIG. 5. Secretion of invertase by *alg* mutants at 36°C. Cells were labeled at 36°C with [³⁵S]methionine for 15 min and then 20 μg of unlabeled methionine per ml was added for 15 min. Spheroplasts were made and centrifuged. The supernatant (S) was decanted and 0.4 ml of spheroplasting solution was added to the pellet (P). Invertase was immunoprecipitated from each fraction and analyzed by NaDodSO₄/polyacrylamide gel electrophoresis. WT is wild type; all other strains contain the indicated mutations. Arrow indicates the position of cytoplasmic invertase.

crease in the mobility of the endo H-treated protein on NaDodSO₄/polyacrylamide gels. Some of the protein-linked oligosaccharides were elongated by outer chain addition. However, at least half of *alg1-1* invertase contained only the amount of glycosylation seen in the *alg1-1 sec18-1* double mutant, indicating that much of the protein contained no outer chain residues. At 26°C *alg1-1* invertase was indistinguishable from wild-type invertase.

The *alg2-1* mutant accumulated lipid-linked Man₁GlcNAc₂ and Man₂GlcNAc₂ at 36°C. Invertase made in *alg2-1 sec18-1* cells contained mainly endo H-resistant oligosaccharides, suggesting that one or both of these species may be transferred to protein. *alg2-1 sec18-1* invertase also contained a small amount of endo H-sensitive oligosaccharides. However, the largest species appeared to possess less than half of the carbohydrate content of *sec18-1* invertase. Only a minor fraction of these chains were elongated in *alg2-1* cells. At 26°C, *alg2-1* invertase still contained endo H-resistant oligosaccharides. Although some of the *alg2-1* invertase remained underglycosylated, many more of the chains were elongated at 26°C than 36°C. This increased amount of outer chain is evidently sufficient for cell viability, although this strain does grow more slowly at 26°C than the other *alg* mutants.

The *alg3-1* mutant accumulated lipid-linked Man₅GlcNAc₂. Although some of the oligosaccharides on *alg3-1 sec18-1* invertase were sensitive to endo H digestion, the majority were resistant. The most likely interpretation of this result is that Man₅GlcNAc₂ was transferred to protein. Interestingly, these

FIG. 6. (A) Structure of protein-linked $\text{Man}_9\text{GlcNAc}_2$ and the site of attachment of outer chain residues. (B) Structure of $\text{Man}_5\text{GlcNAc}_2$ and the proposed site of attachment of outer chain residues in *alg3-1*. All anomeric linkages have the α -configuration, except for the trisaccharide unit $\text{Man} \xrightarrow{\beta 1,4} \text{GlcNAc} \xrightarrow{\beta 1,4} \text{GlcNAc}$. M, Man; N, GlcNAc; $(\text{M})_n$, outer chain mannose residues.

oligosaccharides were elongated and remained endo H resistant. This result is consistent with the known specificity of endo H and the direction of outer chain synthesis. $\text{Man}_5\text{GlcNAc}_2$ has the structure shown in Fig. 6B (5, 6). Addition of an $\alpha 1,3$ -linked mannose residue to the $\alpha 1,6$ -linked mannose residue is known to be sufficient to make the molecule endo H sensitive. Indeed, the pentasaccharide $\text{Man} \xrightarrow{\alpha 1,3} \text{Man} \xrightarrow{\alpha 1,6} \text{Man} \xrightarrow{\beta 1,4} \text{GlcNAc}_2$ is sensitive to endo H digestion (15, 16). Outer chain synthesis in wild-type cells occurs by the addition of $\alpha 1,6$ -linked residues to the terminal $\alpha 1,6$ -linked mannose residue of $\text{Man}_8\text{GlcNAc}_2$ (17), which is generated by processing of the precursor oligosaccharide (Fig. 6A). By analogy, elongation of $\text{Man}_5\text{GlcNAc}_2$ could occur by addition of mannose residues to the terminal $\alpha 1,6$ -linked mannose of $\text{Man}_5\text{GlcNAc}_2$ (Fig. 6A). We would suggest that this addition is not sufficient to cause endo H sensitivity in the absence of the $\alpha 1,3$ -linked residue. Our model of outer chain synthesis in *alg3-1* cells requires that the $\alpha 1,3$ -linked mannose is the sole determinant of endo H specificity. Structural characterization of *alg3-1* oligosaccharides will be necessary to prove this hypothesis.

The *alg4-9* mutant accumulated lipid-linked oligosaccharides ranging from $\text{Man}_1\text{GlcNAc}_2$ to $\text{Man}_8\text{GlcNAc}_2$ at 36°C . This pattern of oligosaccharides cannot be easily explained by a single block in the synthesis of the lipid-linked precursor oligosaccharide. Although *alg4-9* cells synthesized oligosaccharides larger than $\text{Man}_5\text{GlcNAc}_2$, apparently none of these were transferred to protein because *alg4-9* invertase contained no endo H-sensitive carbohydrate. No oligosaccharide chains were elongated at 36°C . In addition, *alg4-9* was the only *alg* mutation that appeared to block secretion of invertase. All of these facts suggest that the primary defect in *alg4-9* cells may not be in lipid-linked oligosaccharide assembly. Rather, this phenotype may be a secondary consequence of a mutation that also affects the transfer of oligosaccharides to protein and secretion of proteins from the cell. *alg4-9* cells synthesized fully glycosylated invertase at 26°C .

Both *alg5-1* and *alg6-1* cells accumulated lipid-linked $\text{Man}_9\text{GlcNAc}_2$. This oligosaccharide from each of the mutants was digested to $\text{Man}_1\text{GlcNAc}_2$ by α -mannosidase, demonstrating that it does not contain any glucose residues (18). Finding mutations in two complementation groups that blocked the addition of the first glucose residue was expected. The donor of glucose residues in precursor oligosaccharide synthesis is dolichol phosphate (Dol-P) glucose, which is made from UDP-Glc and Dol-P (reviewed in ref. 19). Therefore, mutations affecting either the enzyme that synthesizes Dol-P-Glc or the enzyme that uses Dol-P-Glc as substrate and adds the first glucose res-

idue to $\text{Man}_9\text{GlcNAc}_2$ would cause accumulation of $\text{Man}_9\text{GlcNAc}_2$. Biochemical characterization of these mutants has shown that *alg5-1* is blocked in Dol-P-Glc synthesis and *alg6-1* is blocked in the transfer of glucose from Dol-P-Glc to $\text{Man}_9\text{GlcNAc}_2$ (18). Both of these mutants transfer $\text{Man}_9\text{GlcNAc}_2$ to protein (18). Like *sec18-1* invertase, *alg5-1 sec18-1* and *alg6-1 sec18-1* invertase migrated in discrete bands. However, *alg5-1 sec18-1* and *alg6-1 sec18-1* invertase migrated faster than *sec18-1* invertase, indicating that fewer sites are glycosylated in these *alg* mutants. On average, *alg5-1 sec18-1* invertase appeared to contain three less oligosaccharide chains and *alg6-1 sec18-1* invertase appeared to contain four less oligosaccharide chains than *sec18-1* invertase. This result suggests that $\text{Man}_9\text{GlcNAc}_2$ is transferred to protein less efficiently than $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$. As expected, the core oligosaccharides in these mutants were processed by the addition of outer chain residues.

The results presented here demonstrate that oligosaccharides other than $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$ can be transferred to protein when synthesis of the precursor oligosaccharide is blocked. In some cases these aberrant protein-linked oligosaccharides are elongated by the addition of outer chain residues. This processing of oligosaccharides is necessary for cell viability but, in general, appears not to be essential for glycoprotein secretion.

Note Added in Proof. Recent experiments demonstrate that *alg4-9* is allelic to *sec53-6*, which was obtained as a temperature-sensitive mutation that blocks secretion (20). *alg4-9* failed to complement *sec53-6* for growth at 36°C . In addition, *sec53-6* cells grew at 36°C after transformation with a 2- μm plasmid carrying the *ALC4* gene. Ferro-Novick *et al.* (20) conclude that the *SEC53* gene product is required for the translocation of proteins into the endoplasmic reticulum.

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