Two highly conserved eukaryotic gene products of unknown function showing homology to glycosyltransferases involved in the second steps of bacterial peptidoglycan (Murg) and capsular polysaccharide (Cps14f/Cps14g) biosynthesis have been identified in silico. The amino acid sequence of the eukaryotic protein that is homologous to the lipid acceptor- and membrane-associating N-terminal domain of Murg and the Cps14f β4-galactosyltransferase enhancer protein is predicted to possess a cleavable signal peptide and transmembrane helices. The other eukaryotic protein is predicted to possess neither transmembrane regions nor a signal peptide but is homologous to the UDP-sugar binding C-terminal domain of Murg and the Cps14g β4-galactosyltransferase. Both the eukaryotic proteins are encoded by essential genes in Saccharomyces cerevisiae, and down-regulation of either causes growth retardation, reduced N-glycosylation of carboxypeptidase Y, and accumulation of dolichyl-PP-GlcNAc. In vitro studies demonstrate that these proteins are required for transfer of [3H]GlcNAc from UDP-[3H]GlcNAc onto dolichyl-PP-GlcNAc. To conclude, two gene products showing homology to bacterial glycosyltransferases are required for the second step in dolichyl-PP-oligosaccharide biosynthesis.

A long-standing problem in the study of lipid-linked oligosaccharide (LLO, GlcMAn9GlcNAc2-PP-dolichyl) biosynthesis concerns the identity of the enzyme that undertakes the second step of this process (1, 2). Because many aspects of eukaryotic N-glycosylation have evolved from prokaryotic glycosylation pathways (3), knowledge of the latter processes is useful for understanding of mammalian protein glycosylation.

Protein N-glycosylation occurs by the transfer of an oligosaccharide from LLO onto proteins and is initiated by UDP-GlcNAc dolichyl phosphate:N-acetylglucosamine-1-phosphate transferase (DPAGT1). Sequence data show that this enzyme belongs to a family of sugar phosphate transferases that act on phospholipid acceptors to initiate glycolipid synthesis in bacterial peptidoglycan, liposaccharide, exosaccharide, and capsular polysaccharide pathways (4, 5). A practical consequence of these facts is that the potent antibiotic tunicamycin is not clinically useful because it blocks the first step of both the eukaryotic LLO (6) and bacterial peptidoglycan pathways (7).

The second step in peptidoglycan biosynthesis is carried out by UDP-GlcNAc undecaprenyl-PP-MurNAc pentapeptide:N-acetylgalactosaminyltransferase (Murg, 8–10), which has been crystallized (11) and is now a target for antibiotic design (12, 13). In analogous fashion, the second step of the eukaryotic LLO pathway entails conversion of dolichyl-PP-GlcNAc to dolichyl-PP-GlcNAc2 by a poorly characterized N-acetylgalactosaminyltransferase (UDP-GlcNAc dolichyl-PP-GlcNAc:N-acetylgalactosaminyltransferase, L-0AGT, 14–18). A structural relationship between bacterial Murg and LLO-NAGT has not been reported, but such a finding would be of importance for our understanding of the origins and mechanisms of eukaryotic protein glycosylation and would be critical for the current projects dedicated to the design of clinically useful antibiotics that target steps in bacterial cell wall glycoconjugate biosynthesis. Finally, although a congenital disorder of glycosylation caused by a deficiency in LLO-NAGT has not yet been reported, such a finding would be of importance for understanding of the origins and mechanisms of eukaryotic protein glycosylation and would be critical for the current projects dedicated to the design of clinically useful antibiotics that target steps in bacterial cell wall glycoconjugate biosynthesis. Finally, although a congenital disorder of glycosylation caused by a deficiency in LLO-NAGT has not yet been reported, such a finding would be of importance for understanding of the origins and mechanisms of eukaryotic protein glycosylation and would be critical for the current projects dedicated to the design of clinically useful antibiotics that target steps in bacterial cell wall glycoconjugate biosynthesis.
which the promoters of the YBR243c (ALG7), YBR070c, and YGL047w open reading frames are individually replaced by a doxycycline-repressible (Tet-O) promoter (Open Biosystems, Huntsville, AL), and the parental strain R1158 (control cells) were grown in YPD medium at 30 °C. Stationary precultures of the different Tet-O strains were diluted (A600 nm = 0.1) in YPD containing 1–10 µg/ml doxycycline. Culture progression was monitored by turbidimetry during 23 h. Cells were then harvested and resuspended (A600 nm = 0.1) in the same concentrations of drug before cultivating for a further 23 h. A third passage was performed in this manner.

Large Scale Glycolipid Extractions—800 denitometric units of exponentially growing cells were extracted with organic solvents (21, 22). The lower CHCl3 phase and the CHCl3/Methanol/H2O (10:10:3) extract derived from the protein interphase were dried, resuspended in 5 ml of CHCl3/Methanol/H2O (10:10:3), and subjected to DEAE-cellulose chromatography.

**DEAE-cellulose Chromatography—**Yeast lipid extracts were applied to 4 ml DEAE-cellulose (acetate form) columns (22) and eluted with 6 volumes of CHCl3, Methanol, H2O (10:10:3), 6 volumes of CHCl3, Methanol, 5 mM ammonium acetate (10:10:3), and finally 6 volumes of CHCl3, Methanol, 100 mM ammonium acetate (10:10:3).

**Preparation and Incubation of Yeast Microsomes with Nucleotide Sugars—**Microsomes (23) were washed with 50 mM Tris/HCl, pH 7.3, containing 10% w/v glycerol. 400–800 µg of protein were incubated with UDP-[3H]GlcNAc in the above buffer containing 20 mM MgCl2, and where indicated 100 µM Ac-NTY-NH2, in a final volume of 100 µl at 25 °C. In experiments where microsomes were incubated with exogenously glycolipids, the DEAE-cellulose eluates were dried down and taken up in 10 µl of 5% Nonidet P-40. Reactions were terminated by addition of 200 µl of H2O, 600 µl of Methanol, and 900 µl of CHCl3. After shaking and centrifugation, the upper phase was removed and replaced with an equal volume of fresh upper phase. By repeating this procedure twice, the lower CHCl3 phase was washed before being assayed by scintillation counting.

**Thin Layer Chromatography Procedures—**Glycolipids were separated on silica-coated aluminum sheets that were developed in CHCl3/Methanol/H2O, 65:25:4, for 8 h (TLC system A). Sugars released from glycolipid by mild acid hydrolysis (21) were resolved by thin layer cellulose-coated plastic sheets in pyridine/ethyl acetate/water/acetic acid, 5:5:3:1 for 7–8 h (TLC system B, Ref. 21). Radioactive components were visualized by fluorography.

**Galactosyltransferase Assay—**Sugars released from glycolipids were resolved on a Biogel-P2 column (125 × 0.7 cm) developed in 100 mM acetic acid. Fractions corresponding to those of standard GlcNAc and di-N-acetylchitobiose (GlcNAc2) were collected separately and assayed for these two sugars in a reaction volume of 50 µl containing 20 mM NaHCO3, pH 8.5, 20 mM MnCl2, 0.25 µCi UDP-[3H]GlcNAc, and 5 µU bovine milk galactosyltransferase. After 3 h at 37 °C the mixtures were applied to columns of Ag 1-X2 and AG 50-X2, and the water eluates and washes were collected and subjected to scintillation counting.

**Western Blot—**Cell extracts (23) were resolved on 10% SDS-acrylamide gels and blotted onto nitrocellulose membranes. The anti-carboxypeptidase Y (CPY) monoclonal antibody was detected using goat anti-mouse conjugated with horseradish peroxidase.

### RESULTS

**Identification of a Highly Conserved Eukaryote Gene Product of Unknown Function Similar to the C-Terminal Domain of E. coli Murg—**DPAGT1, the enzyme responsible for the first step in the biosynthesis of LLO, has weak homology to UDP-MurNAc pentapeptide:undecaprenyl-P MurNAc(pentapeptide)-1-phosphate transferase (Mray), which catalyzes the formation of undecaprenyl-PP-MurNAc pentapeptide (4, 5), the first lipid linked intermediate in the biosynthesis of bacterial peptidoglycan (Fig. 1). The glycosyltransferases responsible for the second steps of these related pathways may also display similarities because as well as employing a common activated sugar donor the two lipid-linked acceptor molecules share common structural features (Fig. 1). In bacteria, the enzyme Murg transfers GlcNAc from UDP-GlcNAc onto C-4 of the MurNAc residue of undecaprenyl-PP-MurNAc pentapeptide (9) and has been characterized at the atomic level (13). The N- and C-terminal domains of the Murg enzymes belong to Protein Families (Pfam, www.sanger.ac.uk/Software/Pfam/, Ref. 24) PF03033 and PF04101, respectively. While examining PF04101, we noted that as well as containing 176 proteins corresponding to various bacterial Murg sequences it contained a group of proteins that possessed only the Murg C-terminal domain. Within this group we noted the presence of a small protein with unknown function that is highly conserved in yeast, plants, insects, worms, and mammals. To learn more about this protein, the human, toad, and yeast sequences were submitted to a fold recognition server (25) to detect structural features in common with other proteins present in the data bases. Irrespective of the organization of origin, the highest significant consensus values were obtained with E. coli Murg (Protein Data Bank entry 1F0K) as template. Murg comprises N- and C-terminal domains containing a Rossmann fold αβ open sheet structure (11). An alignment of the primary and secondary structural characteristics of Murg with those predicted for YGL047w-like sequences is illustrated in Fig. 2 (lower panel). The YGL047w-like sequences begin with a conserved region that maps onto...
Murg G-loop 3, which is involved in binding the α phosphate of UDP-GlcNAc (11). Importantly, the YGLO47w-like sequences also contain a conserved motif that maps onto a region of Murg (Cβ4-Cα4-Cβ5-Cα5) that contains a consensus sequence for UDP-glucuronosyltransferases (11). YGLO47w-like sequences are predicted to possess neither an N-terminal signal peptide nor transmembrane spanning regions. As all the proteins known to interact with dolichyl-PP-sugars possess several transmembrane regions, the above observations suggested that the YGLO47w gene product may not act efficiently on a highly lipophilic acceptor alone. Accordingly, we looked in PF03033 for mammalian proteins containing only the N-terminal domain of Murg, however no such sequences were apparent.

Identification of Second Highly Conserved Eukaryotic Gene Product of Unknown Function That Is Similar to the N-terminal Domain of E. coli Murg—Important information bearing on polypeptides corresponding to only the C-terminal domain of Murg derives from studies on bacterial liposaccharide, capsular saccharide, and exopolysaccharide synthesis, which have striking similarities to LLO and peptidoglycan biosynthesis (Fig. 1). In fact, the cps14g gene product that is involved in the second step of bacterial capsular polysaccharide biosynthesis (26) is a 167-residue β-galactosyltransferase displaying similarities to the C-terminal domain of Murg (Fig. 2, lower panel), but its activity is greatly enhanced when assayed in the presence of the 149-residue cps14f gene product (26), which shows homology to the N-terminal domain of Murg (Fig. 2, upper panel). Accordingly, we searched the data banks for yeast, plant, and mammalian sequences showing homology to the cps14f gene product. A yeast gene, YBR070c, along with several plant and mammalian homologues were identified (Fig. 2, upper panel). This protein was submitted to the fold recognition server as described above, and irrespective of the organism of origin, the highest significant consensus values were again obtained with E. coli Murg (Protein Data Bank code 1F0K) as template. The YBR070c-like sequences are predicted to possess a hydrophobic N-terminal signal peptide and 1–2 other transmembrane helices that are not present in Murg (Fig. 2, upper panel).

YBR070c and YGLO47w Are Required for N-Glycosylation in S. cerevisiae—As YBR070c and YGLO47w are essential for growth in S. cerevisiae, their functions were examined in YGLO47wDR and YBR070cDR strains in which each gene is under the control of a doxycyclin-repressible promoter (20). These cells, along with ALG7DR cells (in S. cerevisiae the DPATG1 gene is called ALG7, Ref. 27, Fig. 1) and control cells (see “Experimental Procedures”), were grown in the presence of different concentrations of doxycyclin. The growth curves shown in Fig. 3 demonstrate that all except the control cells display reduced growth rates when grown with drug. This
effect was only observed during a second growth passage and was maximal after a third passage in the presence of 1–10 μg/ml doxycyclin. Many yeast strains deficient in enzymes of the LLO pathway generate CPY forms bearing reduced numbers of N-glycans, and it was noted that the distribution of LLO species was the same in both YGL047wDR and control cells (data not shown). These data indicate that the observed block in glycoprotein biosynthesis (Fig. 3) occurs at an early step, before the addition of mannose to the LLO. A similar experiment in which cells were pulse radiolabeled with [6-3H]GlcNAc for 8 min revealed that the ratio of LLO bearing GlcNAc and GlcNAc$_2$ was higher in YGL047wDR cells (0.65) than that (0.17) observed in the control cells. Although this result indicates a potential block in the addition of GlcNAc onto dolichyl-PP-GlcNAc, the low incorporation of radioactivity into these LLO rendered further analysis by this approach difficult.

The YGL047w and YBR070c Genes Products Are Required for the Synthesis of Dolichyl-PP-GlcNAc$_2$—When incubated with UDP-[3H]GlcNAc, microsomes derived from the YGL047wDR and YBR070cDR strains incorporate 30–50% less [3H]GlcNAc into glycolipids than their wild-type-derived counterparts (Fig. 4A, left panel). After fractionating the glycolipids by ion-exchange chromatography, it was noted that [3H]GlcNAc incorporation into neutral or positively charged (Unbound) species was the same in the three microsome populations (Fig. 4A, middle panel). By contrast, elution of the columns with 100 mM NH$_4$Ac, a procedure known to displace dolichyl-PP-oligosaccharides from this ion exchanger (22), indicated that YGL047wDR and YBR070cDR microsomes incorporated only 25–30% the amount of radioactivity into these glycolipids when compared with control membranes (Fig. 4A, right panel). In a similar experiment (data not shown) microsomes derived from ALG7DR cells were found to incorporate only 10% of control levels of radioactivity associated with these negatively charged glycolipids. TLC of the glycolipid fractions that did not bind to DEAE-cellulose revealed a single component that exhibited behavior that was similar irrespective of the microsome population from which it was derived (Fig. 4B, Unbound). Resolution of the negatively charged glycolipids revealed the presence of two major components (Fig. 4B, 100 mM NH$_4$Ac) in control cell-derived microsomes, but much reduced quantities of the slower migrating species were present in microsomes from the YGL047wDR and YBR070cDR strains. Furthermore, incorporation of radiolabel into both these components was sharply reduced in ALG7DR membranes. Separation of the free sugars released from these glycolipids by mild acid hydrolysis (Fig. 4B, 100 mM NH$_4$Ac after HCl) revealed GlcNAc and GlcNAc$_2$ to be present. Indeed, for all incubations the relative amounts of these two sugars were found to reflect the distribution of the two major glycolipid species. It was also noted (data not shown) that these two glycolipid species remained intact after saponification (0.1N NaOH for 15 min at 37 °C). Together, these data demonstrate that the glycolipids with $R_f$ values of 0.25 and 0.14 are the DPAGT1 (alg7p)-dependent species dolichyl-PP-GlcNAc and dolichyl-PP-GlcNAc$_2$, respectively, and that YGL047wDR and YBR070cDR membranes are defective in generating the latter species.

Dolichyl-PP-GlcNAc Accumulates in YGL047wDR and YBR070cDR Strains—If YGL047w and YBR070c encode proteins required for dolichyl-PP-GlcNAc biosynthesis, YGL047wDR- and YBR070cDR-derived microsomes would be expected to accumulate dolichyl-PP-GlcNAc, but data shown in Fig. 4B indicate that under our microsome radiolabeling conditions this may not be the case. It is known that the formation of dolichyl-PP-GlcNAc by DPAGT1 is subject to product inhibition.
DPAGT1-mediated [3H]GlcNAc addition onto dolichyl-P. LLOs, the B(4) and di-N-acetylchitobiose (GN2) formations of standard GlcNAc (wild type, YBR070cDR, YGL047wDR, and ALG7DR yeast cells would be expected to rescue both dolichyl-PP-GlcNAc2 YGL047wDR and YBR070cDR strains, glycolipids from these PP-GlcNAc. If dolichyl-PP-GlcNAc does indeed accumulate in the accumulation of a glycolipid bearing the hallmarks of dolichyl-phosphate-N-acetylglucosaminyltransferase, which is predicted to possess four transmembrane regions but is more readily solubilized than yeast membranes derived from YGL047wDR and YBR070cDR cells. Furthermore, as shown in Fig. 5D, this increase in radioactivity could be accounted for by the increase of [3H]GlcNAc incorporation into dolichyl-PP-GlcNAc2. Finally, as demonstrated in Fig. 5E, glycolipids purified from YGL047wDR and YBR070cDR but not control cells were able to promote efficient glycosylation of the acceptor tripeptide. Together these results demonstrate the presence of striking dolichyl-PP-GlcNAc accumulations in YGL047wDR and YBR070cDR cells.

**DISCUSSION**

Data bank searches revealed the presence of two genes of unknown function that are highly conserved in eukaryotes and essential for growth in *S. cerevisiae*. Our results demonstrate that down-regulation of these genes causes growth retardation and CPY hypoglycosylation in yeast. This phenotype is often observed in yeast possessing deficiencies in early acting enzymes of the LLO biosynthetic pathway. Glycoprotein hypoglycosylation can be caused by either deficiencies in LLO biosynthesis or transfer of the oligosaccharide from LLO onto protein (1). We were unable to pinpoint the defective step in glycoprotein biosynthesis in [2-3H]mannose-radiolabeled YGL047wDR cells. The observed increase in [2-3H]mannose incorporation into both LLO and N-glycans in this strain was surprising in view of the CPY hypoglycosylation results. In fact, we have often noted a similar occurrence in skin biopsy fibroblasts from patients with type 1 congenital disorder of glycosylation. Despite well described blockages in LLO biosynthesis, there is often an increased incorporation of [2-3H]mannose into N-glycans. This phenomenon might be explained by changes in pool sizes of the various activated mannose intermediates that are required for N-glycosylation. By contrast, pulse metabolic radiolabeling of the YGL047wDR strain with [3H]GlcNAc suggested that these cells were unable to efficiently add the second GlcNAc residue onto growing LLO.

We went on to demonstrate that both YGL047wDR and YBR070cDR cells accumulate dolichyl-PP-GlcNAc and that membranes derived from these cells have strikingly reduced capacities to transfer [3H]GlcNAc from UDP-[3H]GlcNAc onto this glycolipid. Taken together, our results show that the YGL047w and YBR070c DR- and YBR070c DR-derived microsomes reduce DPAGT1-mediated [3H]GlcNAc addition onto dolichyl-P. LLOs were therefore extracted from the different cell populations and purified by ion-exchange chromatography as described above. As bovine milk galactosyltransferase transferred galactose (Gal) from UDP-galactose onto GlcNAc and GlcNAc2, with different efficiencies (19), sugars released from the negatively charged glycolipids by mild acid hydrolysis were separated on Bio Gel P2 prior to quantitation using bovine milk galactosyltransferase and UDP-[14C]galactose. As shown in Fig. 5, A and B, the YGL047wDR and YBR070cDR strains reveal striking accumulations of the disaccharide Galβ4GlcNAc indicating accumulation of a glycolipid bearing the hallmarks of dolichyl-PP-GlcNAc. If dolichyl-PP-GlcNAc does indeed accumulate in YGL047wDR and YBR070cDR strains, glycolipids from these cells would be expected to rescue both dolichyl-PP-GlcNAc2 synthesis and peptide glycosylation in ALG7DR membranes, which are unable to generate dolichyl-PP-GlcNAc or glycosylate peptides efficiently. LLOs were extracted from the different cell populations and purified by ion-exchange chromatography as described above prior to being incubated with ALG7DR membranes, UDP-[3H]GlcNAc, and the tripeptide, Ac-NYT-NH2, that contains the N-glycosylation consensus sequence. Fig. 5C shows striking dose-dependent increases in radiolabel incorporation into crude glycolipids when incubations are performed with glycolipids derived from YGL047wDR and YBR070cDR but not control cells. Furthermore, as shown in Fig. 5D, this increase in radioactivity could be accounted for by the increase of [3H]GlcNAc incorporation into dolichyl-PP-GlcNAc2. Finally, as demonstrated in Fig. 5E, glycolipids purified from YGL047wDR and YBR070cDR but not control cells were able to promote efficient glycosylation of the acceptor tripeptide. Together these results demonstrate the presence of striking dolichyl-PP-GlcNAc accumulations in YGL047wDR and YBR070cDR cells.

**FIG. 4.** Glycolipid biosynthesis in microsomes derived from wild type, YBR070cDR, YGL047wDR, and ALG7DR yeast strains. A, microsomes were incubated with UDP-[3H]GlcNAc, and after solvent extraction [3H]GlcNAc incorporation into chloroform-soluble components (CHCl3) was quantitated (left panel). This material was fractionated on DEAE-cellulose yielding unbound glycolipids (middle panel, Unbound) and negatively charged species characteristic of dolichyl-linked oligosaccharides (right panel, 100 mM NH4Ac after HCl). B, these fractions (upper panel, Unbound; middle panel, 100 mM NH4Ac) were examined using TLC system A. After mild acid hydrolysis of the charged glycolipids, the released sugars were examined in TLC system B (lower panel, 10 mM NH4Ac after HCl). Whereas the migration positions of standard GlcNAc (GN) and di-N-acetychitobiose (GN2) are indicated to the left of the lower chromatogram, the migration distance of the predominant species relative to the solvent front (Rf) is indicated to the left of the middle and upper chromatograms.
and Gal incubated with membranes derived from ALG7DR cells, UDP-[3H]Glc-galactosyltransferase and the CHCl₃, MeOH, 100 mM NH₄Ac (10:10:3) eluate YBR070cDR and YGL047wDR cells. GlcNAc₂ (GN₂) and rated on a Biogel P2 column calibrated with standard GlcNAc (was subjected to mild acid hydrolysis. The released sugars were separated and these sugars were pooled separately and assayed for GlcNAc and Glc-


tide chains encoded by different genes (26). In one experiment the N- and C-terminal domains of the former type of enzyme (Fig. 1, Gelk) have been dissociated and expressed where the N- and C-terminal domains of the former type of enzyme. ADP-glucose pyrophosphorylase is a single polypeptide whose two domains are inactive when expressed separately (30). However, when the domains are co-expressed as separate polypeptides the two domains associate tightly, and enzyme activity is achieved (30). Although the cps14f and cps14g gene products are required for efficient β4-galactosyltransferase activity in S. pneumoniae it is not known whether or not these two proteins form a complex (26). Inspection of genome wide protein-protein interaction experiments in S. cerevisiae (Yeast Research Center, University of Washington) do not reveal an unambiguous complex between the YGL047w and YBR070c gene products, but in at least one experiment when the former protein is TAP-tagged at its C terminus and used as bait to detect interacting proteins, the YBR070c gene product appears second in a list of “hits” (31). Although we do not know whether or not these two proteins do in fact associate, primary and secondary structure alignments show that together they possess the main structural features that are thought to be important for the functioning of the E. coli Murg glycosyltransferase (11). The C-terminal domain of Murg contains a peptide sequence that is conserved in the UDP-glucuronosyltransferase family, and a glycine rich loop (G-loop) thought to be involved in binding the α phosphate of UDP-GlcNAc. Both of these regions map onto highly conserved domains of the YGL047w peptide sequences. The Murg N-terminal domain contains two glycine-rich loops (G-loops 1 and 2, Ref. 11) that are thought to bind the lipid acceptor (undecaprenyl-PP-MurNAc), and Fig. 2 indicates that these loops map onto conserved G-containing sequences in the YBR070r-like sequences. In view of the N-ethylmaleimide sensitivity of LLO-NAGT (14), it is of interest to note that the YBR070c gene product-like sequences contain a conserved cysteine residue in a region that corresponds to a region close to G-loop 2 of Murg. In addition to these structural features the YBR070c gene product is predicted to possess a signal peptide and transmembrane helices that are not predicted to occur in the bacterial Murg and cps14f sequences. The second transmembrane region of the YBR070c-associated sequences corresponds to the third α-helix of the Murg N-terminal domain (Nα3) that contains both hydrophobic and positively charged amino acids thought to be involved in docking the bacterial protein to the cell membrane (11).

In summary, we have identified two gene products involved in the second step of LLO biosynthesis in S. cerevisiae. Although it is not understood how the YGL047w and YBR070c encoded proteins function, bioinformatics information predicts that the proteins possess features required of a UDP-GlcNAc dependent N-acetylglucosaminyltransferase capable of acting on dolichyl-PP-GlcNAc. This information provides insight into the evolutionary origin and mechanism of a key step in the eukaryotic N-glycosylation pathway, and whatever the precise function of these two proteins in this reaction, mutations in the human orthologs of these genes may underlie as yet undescribed subtypes of type I congenital disorder of glycosylation.

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Note Added in Proof—On the basis of the results presented in this article we suggest that the S. cerevisiae genes, YGL047w and YBR070c, be called ALG13 and ALG14, respectively.

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Two Proteins Homologous to the N- and C-terminal Domains of the Bacterial Glycosyltransferase Murg Are Required for the Second Step of Dolichyl-linked Oligosaccharide Synthesis in Saccharomyces cerevisiae

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Two proteins homologous to the N- and C-terminal domains of the bacterial glycosyltransferase Murg are required for the second step of dolichyl-linked oligosaccharide synthesis in *Saccharomyces cerevisiae*.

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**Page 9238, Fig. 2:** Due to an inadvertent error, the sequence alignments shown in Fig. 2 were printed too small to be viewed conveniently. The enlarged figure is shown on the following page. The last line in the Fig. 2 legend should be changed to read: “In the alignment of the YGL047w-related sequences, the heavy dashed blue line indicates the region corresponding to a peptide motif that is conserved in the UDP-glucuronosyltransferase family.”
FIG. 2

Additions and Corrections