Golgi GDP-mannose Uptake Requires Leishmania LPG2
A MEMBER OF A EUKARYOTIC FAMILY OF PUTATIVE NUCLEOTIDE-SUGAR TRANSPORTERS*

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The synthesis of glycoconjugates within the secretory pathway of eukaryotes requires the provision of luminal nucleotide-sugar substrates. This is particularly important for eukaryotic microbes such as Leishmania because they must synthesize considerable amounts of extracellular and cell surface glycoconjugates that play significant roles in the infectious cycle. Here we used properly oriented sealed microsomes to characterize luminal uptake of GDP-Man in Leishmania donovani. In this system, GDP-Man uptake was saturable with an apparent Km for GDP-Man of 0.3 μM and facilitated its use as a donor substrate for lipophosphoglycan (LPG) synthesis. A lpg2 deletion mutant showed loss of GDP-Man but not UDP-Gal uptake, which was restored by introduction of the gene LPG2. Immunoelectron microscopy localized an active, epitope-tagged LPG2 protein to the Golgi apparatus. Thus, LPG2 is required for nucleotide-sugar transport activity and probably encodes this Golgi transporter. LPG2 belongs to a large family of eukaryotic genes that potentially encode transporters with different substrate specificities and/or cellular locations. In the future, the amenability of the Leishmania system to biochemical and genetic manipulation will assist in functional characterization of nucleotide-sugar transports from this and other eukaryotes. Furthermore, since LPG2 plays an important role in the Leishmania infectious cycle and mammalian cells lack a Golgi GDP-Man transporter, this activity may offer a new target for chemotherapy.

Eukaryotic glycosylation mechanisms often require the translocation of dolichol-linked saccharides and/or transport of nucleotide-sugars synthesized in the cytoplasm into the lumen of the appropriate microsomal organelle. A broad array of nucleotide-sugar transporter (NST) activities with differing substrate specificities in the Golgi apparatus or endoplasmic reticulum have been reported (1). Known NST activities include those for UDP-Gal, UDP-Glc, UDP-GlcNac, UDP-glucuronic acid, UDP-xylose, UDP-GalNac, GDP-fucose, GDP-Man, and CMP-sialic acid (1). Three candidate NST genes have been reported recently: one for the UDP-GlcNac transporter from Kluyveromycyes lactis (2), and the others for the CMP-sialic acid transporter (3) and the UDP-Gal translocator (4) from mammalian cells. These genes are required for NST activity, although it is unknown whether they encode the NST itself. In different organisms and cell types, NSTs are involved in both housekeeping and specialized cellular functions (1). In this work we describe another role for NSTs, in the synthesis of glycoconjugates implicated in pathogenicity.

The protozoan parasite Leishmania synthesizes a variety of abundant complex glycoconjugates, whose expression permits the parasite to persevere in hostile environments throughout its life cycle in the sand fly and mammalian phagolysosome (5, 6). One of these is lipophosphoglycan (LPG), the major cell surface glycoconjugate of the promastigote (insect stage) form of the parasite. In L. donovani, LPG contains four domains: a lipid anchor (1-O-alkyl-2-lyso-phosphatidylinositol), a glycan core with the structure Gal[α(1,6)Gal[α(1,3)Gal[β(1,3)]Glc(α1-P04)−6][Man(α1,3)Man(α1,4GlcN(α1,6)], a series disaccharide-phosphate repeating units, and a cap containing one of several small oligosaccharide-phosphates containing β-galactose and α-mannose (5, 6). The repeats are attached to the C-6 hydroxyl of the distal Gal of the glycan core, and each of the 15−30 disaccharide-phosphate units has the structure [Gal[β1,4][Man(α1-P04)−6] in L. donovani. The repeating unit domain is the most prominent feature of LPG, and repeating units also occur on secreted proteins, most prominently an abundant secreted acid phosphatase, a molecule also implicated in parasite virulence (7–9).

The [Gal(β1,4)Man(α1-P04)−6] repeating units are synthesized by the sequential transfer of mannose 1-phosphate and galactose from their respective nucleotide donors (10, 11). The cellular compartment in which repeating unit synthesis likely occurs is the Golgi apparatus, as monensin treatment inhibits repeating unit attachment to both LPG and the secreted acid phosphatase (12). Since no evidence was obtained for the participation of lipid intermediate carriers for the two sugars in this process (10), the nucleotide-sugar substrates for these reactions need to be transported from their cytosolic site of synthesis to the site of utilization in the Golgi lumen. Thus, due to the large amounts of LPG and related glycoconjugates synthesized by the parasite, considerable demands are placed upon NST activity during the infectious cycle.

One of the attractive features of the LPG system is the ability to identify LPG-deficient mutants (13−15) and then by functional genetic rescue isolate genes involved in LPG biosynthesis (16, 17). Previously we described the C30PO mutant, which shows a complete lack of repeating units on LPG and the secreted acid phosphatase (17). Despite its lpg− phenotype,
crude microsomal preparations of C3PO were able to conduct LPG synthesis, suggesting that the defect in this mutant lay in the assembly or compartmentalization of LPG biosynthesis, rather than in a specific glycosyltransferase activity. The gene LPG2 was shown to be deleted in C3PO, and the predicted LPG2 protein encoded a hydrophobic membrane protein. From these data, we proposed that LPG2 was involved in the transport of a key LPG biosynthetic precursor or activity into a new cellular compartment, presumably the lumen of the Golgi apparatus.

In this paper we describe the development of an in vitro assay to demonstrate intravesicular GDP-Man transport in Leishmania membrane vesicles, and use this system to establish that the Leishmania gene LPG2 is essential for GDP-Man uptake. Sequence data base searches indicate that LPG2 is a member of a large eukaryotic gene family, which is proposed to include members affecting NST activity with different subcellular localization within a large eukaryotic gene family, which is proposed to include members affecting NST activity with different subcellular localization.

**EXPERIMENTAL PROCEDURES**

**Materials**—Materials were obtained as follows. GDP-[1-14C]Man (50 mCi/mmole), UDP-[6-3H]Gal (10.2 Ci/mmole), GDP-[1-14C]fucose (10 Ci/mmole), and CMP-[3H]sialic acid (20 Ci/mmole) were from American Radiolabeled Chemicals; Dulbecco’s modified Eagle’s medium and M199 medium were from Life Technologies, Inc.; thin layer chromatography cellulose plates were from Eastman Kodak Corp.; and AG 1-X8 anion exchange resin was from Bio-Rad. All other chemicals were from Sigma.

**Cell Cultures**—Promastigotes of L. donovani (1S2D) strain were grown at 25 °C in Dulbecco’s modified Eagle’s medium (13). The LPG-defective, L. donovani mutant C3PO (14) was grown in the above medium supplemented with 10% fetal bovine serum. C3PO transfected with either cLHYG cosmids containing Leishmania genomic DNA bearing LPG2 or the expression vector pX63HYG-LPG2/HA (17) were maintained in M199 medium supplemented with 10% fetal bovine serum and hygromycin B (50 μg/ml).

**Isolation of Membrane Vesicles**—Stationary phase L. donovani were harvested, pelleted at 3,000 g for 15 min, washed with phosphate-buffered saline (13), and resuspended in the reaction buffer (10 mM Tris-HCl, pH 7.4, 25 mM sucrose, 1 mM TESK, 1 μg/ml leupeptin, 1.0 μM pepstatin A, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM 2,3-dimercapto-1-propanol). The cells were disrupted by nitrogen cavitation at 1,500 p.s.i. for 25 min at 4 °C. Microsomal vesicles were isolated according to the procedures of Goud et al. (18). Vesicle integrity was determined by measuring the latency of galactosyltransferase-catalyzed transfer of [3H]galactose from UDP-[3H]Gal to GlcNAc (19).

**Standard GDP-Man Transport Assay**—The transport assays were started by mixing 100 μl of vesicle protein (1 mg) with 100 μl of reaction buffer (above) containing GDP-[1-14C]Man (final concentration of 3 μM). After incubation at 28 °C for 5 min, the samples were placed on ice and diluted with 1.5 ml of reaction buffer and applied onto a filtration apparatus (Millipore 1225 Sampling Manifold) containing HA filters (24 mm diameter, 0.45 μm pore size). The filters were washed with 25–30 ml of buffer and radioactivity on the filters was measured by scintillation counting. The amount of GDP-[14C]Man that was nonspecifically bound to the outside of the vesicles was determined by measuring the radioactivity associated with the vesicles at zero time of incubation of vesicles with solute.

**Identification of GDP-Man within Vesicles**—Two methods were used to demonstrate the presence of GDP-[14C]Man within the vesicles following transport. In one method, after a 6-min transport assay, the washed filters were placed into 1 ml of water, sonicated for 15 min at 4 °C, and extracted twice with 5 ml of chloroform/methanol (3:2) in the final proportion of chloroform:methanol:water of 3:2:1. The aqueous phases were combined and desalted by gel filtration on a column of Bio-Gel P2. An aliquot of the desalted sample was applied to a thin layer plate using methanol/formic acid/water (80:15:5) as the solvent system and GDP-mannose as the chemical standard. GDP-Man was detected with silver nitrate and ethanolic NaOH (20). Radioactive samples were scanned by a Bicscan detector.

In a second method, identification of GDP-[14C]Man by chromatography on concanavalin A-Sepharose was performed based on the protocol described by Rush and Waechter (21). Briefly, another aliquot of the desalted sample was dried under a stream of nitrogen, redissolved in 0.1 ml of water, and applied to a column (4 ml) of concanavalin A-Sepharose equilibrated in 15 mM ammonium phosphate, pH 5.5, containing 1 mM CaCl2, 1 mM MgCl2 and 1 mM MnCl2. GDP-[14C]Man was eluted with 50 mM α-methylmannoside in the equilibration buffer.

**Cell-free Biosynthesis Assay of LPG**—In vitro LPG biosynthesis was performed as described by Carver and Turco (10). The microsomal vesicles (1 mg of protein) were incubated with 2 μM GDP-[3H]Man (0.4 μCi), 10 μM UDP-galactose in HEPES buffer (50 mM HEPES, pH 7.4, 25 mM KCl, 5 mM MnCl2, 5 mM MgCl2, 0.1 mM TESK, 1 μg/ml leupeptin, 0.8 mM ATP, 0.5 mM dithiothreitol) at 28 °C for 1 h in a final volume of 250 μl. The reaction was terminated by addition of chloroform/methanol to the incubation mixture to give a final ratio of chloroform/methanol/water (4:8:3) and sonicated. By protocols described elsewhere (10), the membranes were sequentially extracted and the radioactive LPG product was purified and quantitated.

**Immunolocalization of an Epitope-tagged LPG2 Protein**—Immunoelectron microscopy was performed on frozen sections from transfected promastigotes fixed in 1% glutaraldehyde in Hepes saline and infiltrated in polyvinylpyrrolidone/sucrose as described previously (22, 23). Sections were probed with rabbit antibody raised against a synthetic peptide YPYDVPDYASL, corresponding to the influenza hemagglutinin tag, coupled to ovalbumin. The anti-HA antibody was affinity-purified on peptide-Sepharose and used at 1 μg/ml diluted in Hepes saline with 5% fetal bovine serum and 5% goat serum. Binding of primary antibody was visualized by incubation with goat anti-rabbit IgG conjugated to 18 nm gold (Jackson Immunoresearch Laboratories). Controls were conducted with non-transfected promastigotes and by omission of the primary antibody. Under the conditions used background labeling remained below 4% of the specific label.

**RESULTS**

**Development of a GDP-Man Transport Assay in L. donovani**

Properties of the GDP-Man Transport System—L. donovani parasites were disrupted by nitrogen cavitation, centrifuged at 10,000 × g to remove large organelles, and sealed microsomal vesicles were obtained by centrifugation at 100,000 × g. The integrity of the microsomal vesicles was estimated by measuring the latency of galactosyltransferase to proteolytic digestion with trypsin (19). The results from this intactness assay indicated that at least 90% of the vesicles were sealed in the proper orientation (data not shown).

Optimization conditions for transport of GDP-Man into Leishmania microsomal vesicles, it was important to establish that transport had characteristics of a protein carrier-mediated system. GDP-Man transport was: (i) time-dependent with linearity up to 6 min (Fig. 1A), (ii) temperature-dependent with approximately 15% of the activity at 4 °C compared to that obtained at 28 °C, and (iii) protein-dependent with linearity...
from 0.05–0.3 mg (Fig. 1B). As shown in Fig. 2, transport was saturable with an apparent $K_m$ of 0.3 $\mu$M for GDP-Man and a $V_{max}$ of 25 pmol/mg/6 min. Under identical assay conditions, no transport of CMP-NANA was observed whereas the transport of GDP-fucose was approximately 5% the rate compared to GDP-Man. On the other hand, translocation of UDP-Gal into the microsomal vesicles was substantial (detailed below) as would be expected due to its utilization for luminal synthesis of LPG and related glycoconjugates.

The requirement for an intact permeability barrier for GDP-Man transport was demonstrated by showing that uptake of the nucleotide-sugar was abolished when the vesicles were incubated with 0.1% (w/v) Triton X-100 (Table I). Furthermore, in the absence of detergent, GDP-Man transport was completely abrogated by DIDS, a stilbene derivative that has been utilized as an inhibitor of nucleotide-sugar transport into mammalian Golgi vesicles (24, 25). Transport was also inhibited by pretreatment of the microsomal vesicles with protease XIV (Table I), suggesting the presence of an important cytoplasmic transporter activity, which would be expected due to its utilization for luminal synthesis of LPG and related glycoconjugates.

Identification of GDP-Man Transported into Vesicles—To provide direct evidence that GDP-[14C]Man was transported into the lumen of the membrane vesicles, the nature of [14C]mannose-labeled, vesicle-associated material was studied. Following a 6-min incubation of the vesicles with GDP-[14C]Man, the vesicles were washed by filtration and then extracted with organic solvents to isolate LPG and water-soluble solutes. [14C]Man by chromatography on concanavalin A-Sepharose, pretreatment of another aliquot of the sample under mild acid conditions that cleaves the labile nucleotide-sugar resulted in quantitative recovery of radioactivity in the void volume (Fig. 4B). In a key control experiment, only negligible radioactivity (<1% compared to wild type microsomal vesicles) was extractable using microsomal vesicles prepared from LPG-deficient C3PO parasites (discussed below).

Nucleotide-sugar Transport by Microsomal Vesicles from C3PO and LPG2-transfected C3PO Cells

Among the possible roles for LPG2 transporter activity, we considered transport of GDP-Man since Man-P addition to the glycan core is the first step of repeating unit biosynthesis (see Introduction). In these studies we made use of mutants lacking the LPG2 gene (C3PO) and of C3PO to which LPG2 expression had been restored by transfection of a LPG2 expression vector (pX3HYG-LPG2).

First, the relative ability to transport GDP-[14C]Man was assayed in microsomal vesicles. As shown in Table II, vesicles from C3PO cells failed to transport significant amounts of GDP-[14C]Man. This defect, in turn, was responsible for the inability of sealed vesicles to synthesize sizable amounts of LPG in vitro in the absence of Triton X-100 (Fig. 5, lower panel), since without translocation the precursor would be unavailable inside vesicles. Incorporation of radioactive man-
nose from GDP-[14C]Man into LPG could only occur when the vesicles were unsealed with concentrations of at least 0.1% Triton X-100. In contrast, vesicles from wild type cells were able to carry out LPG synthesis using GDP-[14C]Man, in the absence or presence of the detergent (Fig. 5, upper panel), as anticipated with a functional GDP-Man transporter. The higher concentrations of detergent were found to have a partial inhibitory effect on LPG synthesis activity as reported previously (10).

An examination of GDP-[14C]Man transport using vesicles prepared from C3PO cells transfected with LPG2 resulted in restoration of approximately 13% of wild type transport levels (Table II). We reasoned that the low level of transport reflected relatively weak expression of LPG2, which was carried on a large cosmid vector. Since elevated drug pressure is known to increase episomal plasmid copy number (26), the hygromycin B concentration was increased from 50 to 100 and then 250 μg/ml, and the cells grown for several passages. Microsomal vesicles were prepared from these transfectants, and assayed for GDP-[14C]Man transport. As shown in Table II, there was a proportional increase in in vitro GDP-[14C]Man transport activity using vesicles from LPG2-transfected cells selected with elevated amounts of hygromycin B.

Significantly, alterations in GDP-Man uptake were specific, as no alterations in uptake of UDP-[3H]Gal were observed in sealed vesicles from wild type, C3PO, and C3PO cells transfected with the LPG2 gene and maintained in various concentrations of hygromycin B (Table II).
Subcellular Localization of the Protein Encoded by LPG2

In an earlier study (17), an epitope-tagged LPG2 protein was detected in a region of the parasite between the nucleus and the kinetoplast, which is where the parasite Golgi apparatus lies. We used immunoelectron microscopy to confirm whether the LPG2 protein was localized to the Golgi apparatus. The LPG2-HA construct described previously was transfected into the C3PO mutant, where it restored the LPG phenotype (17). By electron microscope-gold immunostaining techniques utilizing an antibody that recognizes the HA tag, particles were observed only in the Golgi cisternae that are located near the flagellar pocket (Fig. 6B). In all trypanosomatids the Golgi is represented as a stack of flattened membraneous vesicles that abut the flagellar pocket (Fig. 6A), which is the sole demonstrated site of secretion of proteins and carbohydrate complexes from these organisms (7, 27). Examination of areas where the vesicles of the Golgi are distended indicate that the majority of the label (the C terminus of the LPG2 protein bearing the HA tag in this construct) is associated with the luminal face of the membrane, (Fig. 6C).

DISCUSSION

A topological need for a transporter of GDP-Man, as well as UDP-Gal, in the Golgi apparatus of Leishmania is understandable, based upon our current knowledge of repeating unit assembly of LPG and other phosphoglycan-containing glycoconjugates. Biosynthesis occurs in the Golgi, and consistent with this finding, the protein encoded by the LPG2 gene was clearly localized by immunoelectron microscopy to the Golgi apparatus with the C terminus of the protein being lumenerally oriented. Other LPG biosynthetic genes have also been localized to the Golgi apparatus (28).

Evidence for the existence of a transporter for GDP-Man in Leishmania resulted from the development of an in vitro transport assay involving sealed microsomal vesicles. GDP-Man transport exhibited the normal properties of a protein-mediated system, including selectivity of solutes and sensitivity to protease. Furthermore, transport was inhibited by DIDS, a commonly used inhibitor of nucleotide-sugar transport in mammalian Golgi vesicles. Of particular importance, GDP-Man was discovered within the sealed Leishmania vesicles following a timed incubation. Once inside, the nucleotide-sugar was used as a substrate for LPG synthesis. Identically prepared vesicles from C3PO cells neither transported the substrate nor incorporated the sugar into LPG unless the vesicles were unsealed with detergent.

In mammalian Golgi vesicles, nucleotide-sugar transporters are classified as antiporters since transport is always coupled to the exit of the corresponding nucleoside monophosphate (25). The GDP-Man transporter in yeast is believed to operate via a coupled antiporter reaction (29). A guanosine diphosphatase has been shown to be required for protein and sphingolipid mannosylation in the Golgi lumen of Saccharomyces cerevisiae, implicating the significance of GDP-Man/GMP exchange (30). In the Leishmania system, coupling of entry of GDP-Man into the Golgi compartment to exit of GMP is not known nor has a guanosine diphosphatase been demonstrated. The latter enzyme may not be necessary in Leishmania because GMP presumably is one product produced in abundance by the mannosyl-phosphate transferase reaction during assembly of the repeat units of LPG (11).

We showed that the Leishmania gene LPG2 is required for transport of GDP-Man into the lumen of the Golgi apparatus. Although we favor the idea that LPG2 encodes the GDP-Man transporter of Leishmania, we cannot rule out the possibility that the LPG2 encoded protein is not the GDP-Man transporter but might act in concert with another protein.
transporter itself, strictly speaking it is possible that LPG2 may be an essential component of a multi-subunit NST transporter complex. Similar reservations pertain to other putative NST genes recently identified (2–4). This issue might be resolved by reconstitution experiments of the protein in artificial liposomes or by expression of the LPG2 gene in a heterologous system, such as mammalian cells, that do not possess a Golgi GDP-Man transporter.

The LPG2 Gene Family—We reported previously that LPG2 showed strong homology to the yeast Golgi protein encoded by VRG4/VAN2 (17, 31). Since that time this gene family has grown to include three more from S. cerevisae, one from K. lactis, and four from Caenorhabditis elegans, all of which form a clearly related group of hydrophilic membrane proteins (Figs. 7 and 8, Table III). This family also shows similarity to the Brassica triose phosphate translocator as well (Figs. 7 and 8, Table III). Moreover, searches of the EST sequence data bases additionally revealed at least two human and one plant gene (Table III). The group termed “NST1” includes 23 different ESTs and yields a contiguous overlap of more than 1323 bases additionally revealed at least two human and one plant gene (Table III). The group termed “NST1” includes 23 different ESTs and yields a contiguous overlap of more than 1323 base pairs, which is sufficient to encode a protein similar in size to LPG2 or its relatives (Table III). Interestingly, the putative CMP-sialic acid and UDP-galactose translocators recently identified in mammalian cells (3, 4) shows little sequence divergence among the descendant species.

![Evolutionary tree depicting relationships among LPG2 and potential NSTs.](image)

**FIG. 8.** Evolutionary tree depicting relationships among LPG2 and potential NSTs. The tree was constructed using amino acid sequences for the genes shown in Table III. The evolutionary tree was constructed with the neighbor-joining algorithm (35) using programs implemented in the DNASTar Lasergene package. The numbers in the abscissa represent the percent amino acid sequence divergence among the descendant species.

Family of related genes known or potentially implicated in NST activity

| Name     | Organism          | Data base                  | Function                          |
|----------|-------------------|---------------------------|-----------------------------------|
| LPG2     | L. donovani       | U26175                    | Golgi GDP-Man transport           |
| VRG4     | S. cerevisae      | L38915                    | Golgi GDP-Man transport?          |
| YEM9     | S. cerevisae      | Z48659                    | Golgi UDP-GlcNAc transport        |
| C52E12.3 | C. elegans        | U50135                    |                                   |
| C50F4.14 | C. elegans        | Z70750                    |                                   |
| MNN2–2   | K. lactis         | U48413                    |                                   |
| YEA4     | S. cerevisae      | U18530                    |                                   |
| C53B4.6  | C. elegans        | Z68215                    |                                   |
| F54ET.6  | C. elegans        | U00607                    |                                   |
| Sc38RCSV.111 | S. cerevisae       | Z67751                    | Triose-phosphate translocator    |
| Plant plastid | B. oleracea          | U13632                    |                                   |
| “NST1” Human | ESTs (including W80625, H65599, W02480) | 23 ESTs | 23 ESTs (including R24922, R36287) |
| “NST2” Human | ESTs (including R24922, R36287) | 4 ESTs | 4 ESTs |
| “NST3” Plant | ESTs (including R24922, R36287) | 4 ESTs | 4 ESTs |

Table III

A family of related genes known or potentially implicated in NST activity

| Name     | Organism          | Data base                  | Function                          |
|----------|-------------------|---------------------------|-----------------------------------|
| LPG2     | L. donovani       | U26175                    | Golgi GDP-Man transport           |
| VRG4     | S. cerevisae      | L38915                    | Golgi GDP-Man transport?          |
| YEM9     | S. cerevisae      | Z48659                    | Golgi UDP-GlcNAc transport        |
| C52E12.3 | C. elegans        | U50135                    |                                   |
| C50F4.14 | C. elegans        | Z70750                    |                                   |
| MNN2–2   | K. lactis         | U48413                    |                                   |
| YEA4     | S. cerevisae      | U18530                    |                                   |
| C53B4.6  | C. elegans        | Z68215                    |                                   |
| F54ET.6  | C. elegans        | U00607                    |                                   |
| Sc38RCSV.111 | S. cerevisae       | Z67751                    | Triose-phosphate translocator    |
| Plant plastid | B. oleracea          | U13632                    |                                   |
| “NST1” Human | ESTs (including W80625, H65599, W02480) | 23 ESTs | 23 ESTs (including R24922, R36287) |
| “NST2” Human | ESTs (including R24922, R36287) | 4 ESTs | 4 ESTs |
| “NST3” Plant | ESTs (including R24922, R36287) | 4 ESTs | 4 ESTs |

Other NSTs

| Name     | Organism          | Data base                  | Function                          |
|----------|-------------------|---------------------------|-----------------------------------|
| CMP-SA-Tr | CHO cells       | Z71268                    | CMP-sialic acid transport         |
| UGT      | Had-1 cells       | D84454                    | UDP-Gal transport                 |

a 1337-bp contig., BLASTX p = 10^{-9} with U00067, Z67751.

b 713-bp contig., BLASTX p = 10^{-10} with U50135.

c 784-bp contig, BLASTX p = 10^{-9} with Z48639.

Within the LPG2 family, the properties of the Kluyveromyces MNN2–2 gene show interesting similarities to those of LPG2; mnn2–2 mutants exhibit decreased uptake of a nucleotide-sugar, UDP-GlcNAc (32), and hydrophilic analysis predicts a very hydrophobic protein with multiple putative transmembrane domains (2). Preliminary data have suggested that yeast *vrg4* mutants have reduced GDP-Man uptake as well. Thus, it seems reasonable that the members of this gene family may share the common feature of involvement in NST. The finding of a large and expanding gene family meshes well with current views, that eukaryotes exhibit numerous and varied NST activities with different substrate specificities in the Golgi apparatus or endoplasmic reticulum. Significantly, the identification of LPG2-related mammalian ESTs will permit rapid cloning of the full-length cDNA, and ultimately, functional characterization of the encoded proteins of mammalian cells (1). Thus, studies of the protozoan *Leishmania* have provided important new insights for the function of genes in other eukaryotic organisms such as *S. cerevisae* and humans. Moreover, the amenability of the *Leishmania* system to biochemical and genetic manipulation and the relative ease in preparing intact vesicles could be exploited in the functional characterization of NST activities from other eukaryotes.

Role of LPG2 in Glycoconjugate Synthesis—Despite their functional and sequence similarities, disruption of *Leishmania* LPG2 has profoundly different consequences than disruption of *S. cerevisae* VRG4 (31, 33). LPG2 deletion mutants are deficient in a very specific biochemical step: the synthesis of the repeating units normally present in LPG and related glycoconjugates (5, 6). Otherwise, *lpg2*−*Leishmania* are completely normal in protein N-glycosylation, secreted or GPI-anchored protein expression and localization, glycolipid profile, and growth rate. In contrast, VRG4 is an essential gene, and partial loss of function mutants show severe defects in protein targeting, glycosylation, Golgi structure, and growth (31). These differences led to the proposal that LPG2 belonged to a pathway of genes related to those of other eukaryotes, but whose members had specialized toward the synthesis of LPG and related
molecules, most of which are believed to play important roles unique to the parasite infectious cycle (5, 6).

The finding that LPG2 participates in what might be viewed as a “housekeeping” function, provision of Golgi luminal GDP-Man, suggests that the postulated specialization evolved, not by duplication and divergence as originally proposed, but in another way. One possibility is that the LPG pathway occurs in a separate compartment, thereby necessitating a dedicated GDP-Man transporter. This seems unlikely as the crude or- ganellar preparations studied here would be expected to contain most if not all intracellular compartments, yet the lpg2- mutant C3PO exhibits essentially no uptake of GDP-Man sug- gestive of a second NST activity. More likely is the possibility that specialization indeed has occurred in the Leishmania secretory pathway, not by gene duplication and divergence, but by differential expression and/or usage. Although eukaryotic cells exhibit numerous and diverse NST activities, most cell types feature only a subset of these (1), as salient example being the lack of GDP-Man uptake activity in mammalian cells. The minor consequences of loss of UDP-GlcNAc uptake in mnn2-2

...in this requirement suggests that Golgi GDP-Man transport may represent a potential target for effective chemotherapeutic intervention. Further characterization and purification of the Leishmania GDP-Man transporter may lead to the identification of specific inhibitors of this pathway in the future.

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Note Added in Proof—Recently, another member of the LPG2 family was reported (GenBank™ Accession number D87449). This human sequence is related to the C-elegans C52E12.3 sequence (32% identity) and illustrates the rapidity of expression of this gene family.

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