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REVIEW

Structure, Functions, and Biosynthesis of Glycoconjugates of *Leishmania* spp. Cell Surface

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Abstract—Cell surface of leishmaniasis causal agent, a parasitic member of Protozoa of *Leishmania* genus, is covered by thick glycocalix consisting of various phosphatidylinositol-anchored molecules. This review deals with the structure and biosynthesis of the main phosphoglycans and glycoproteins of *Leishmania* cell surface, many of which incorporate the rare natural D-arabinopyranose, and the problem concerning the involvement of these molecules in support of *Leishmania* survival during their intricate life cycle is discussed.

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Parasitic Protozoa of the *Leishmania* genus are causal agents of leishmaniasis, and during their whole life they circulate between a blood-sucking sand-fly and humans. Their life cycle consists of two stages: flagellar promastigote in the insect's stomach and flagellum-free amastigote (Leishman–Donovan bodies) in human phagolysosomes [1]. Their unusual mode of life has a pronounced effect on *Leishmania* and defines their cell morphology and biochemistry. An example is the glycocalix of *Leishmania* cell surface, which consists of lipophosphoglycan and various glycosylphosphatidylinositol (GPI)-anchored glycoproteins covering a layer of small glycosylinositol phospholipids (GIPL) [2-6]. The phosphoglycan repertoire on *Leishmania* cell surface is changeable; it changes dynamically together with the protozoon life cycle. Lipophosphoglycan and GPI-anchored proteins are expressed on the promastigote membranes, while their expression in amastigotes significantly decreases, and at this stage GIPL remain the dominant molecules. The change in glycocalix composition helps *Leishmania* in misleading the human and insect protective systems and owing to this in surviving in conditions of constantly changing environment. This review considers structures and biosynthesis of the main molecules of *Leishmania* cell surface, many of which incorporate the rare natural D-arabinopyranose, as well as their biological role in the parasitic protozoon's virulence and survival.

Lipophosphoglycan structure. Lipophosphoglycan is the main molecule of cell surface of *Leishmania* promastigotes and covers the whole cell including the flagellum [7]. It is synthesized by all *Leishmania* species and is necessary for the survival of the protozoon in organisms of both vectors and hosts [1]. The lipophosphoglycan molecule consists of four sites (Fig. 1), three of which, the lipid anchor, glycan core, and the chain of repetitive units (Galβ1,4Manα1-PO_4) are identical in all *Leishmania* species [1, 8]. The fourth site, the oligosaccharide cap as well as side substituents of repetitive units, change depending on the *Leishmania* species and the life cycle stage [8-10]. The lipophosphoglycan lipid anchor consists of phospholipid derivative 1-*O*-alkyl-2-*lyso*-phosphatidyl(myo)inositol with one saturated C_{24-26} aliphatic chain. The glycan core, consisting of non-acetylated glucosamine, two mannose residues, two galactopyranose residues, and of a rare natural galactofuranose residue, is attached to inositol [11]. In *L. donovani*, *L. mexicana*,

Abbreviations: D-Ara, D-arabinose; Gal, D-galactose; GIPL, glycosylinositol phospholipids; GlcN, glucosamine; GPI, glycosylphosphatidylinositol; Man, D-mannose (monosaccharides are in pyranose form unless otherwise stated); PI, phosphatidylinositol.

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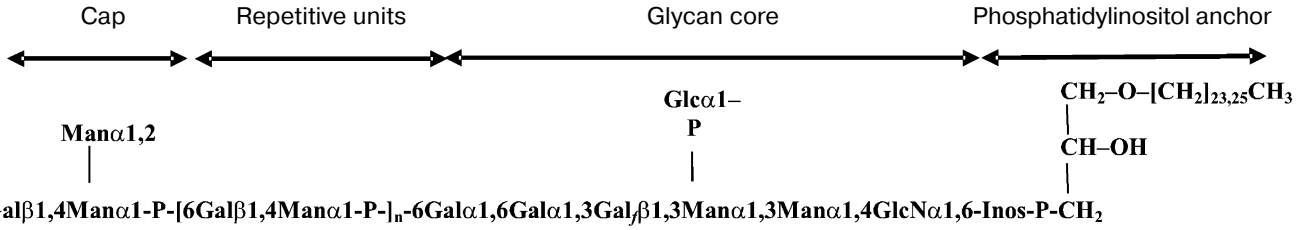


Fig. 1. Structure of *L. donovani* lipophosphoglycan. Gal_f, galactofuranose; P, phosphate.

and in some subspecies of *L. major*, hydroxyl C6 of the second (from reducing terminus) mannose residue is substituted by glucose-1-phosphate via a phosphodiester bond.

A characteristic feature of all known lipophosphoglycan species is the chain of repetitive disaccharide Galβ1,4Manα1-PO_4 [1, 12-15] with species-specific side substituents. Depending on the side chain nature and structure, three types of lipophosphoglycans are distinguished [10]. Type 1 lipophosphoglycan has no side substituents and is found in *L. donovani*. Type 2 lipophosphoglycan is glycosylated at the galactose position C3 in repetitive units. This group is found in *L. mexicana*, *L. major*, and *L. tropica*. Type 3 lipophosphoglycan is specific for *L. aethiopica*; it contains 35% of repetitive units that are mannosylated at position C2 of mannose. The carbohydrate composition of side chains also depends on the *Leishmania* species. In *L. mexicana* they consist of β-D-Glc residues, and in *L. major* of (1–3)-bound β-D-Gal residues terminated by β-D-Ara at certain stages of life cycle. *Leishmania tropica* has the most complexly modified side chains of 19 various monosaccharide residues.

The lipophosphoglycan terminus is capped by a small oligosaccharide consisting of galactose and mannose residues. The cap of *L. major* promastigotes has the simplest structure, Manα1,2Manα1 [16], while lipophosphoglycans of *L. donovani* and *L. mexicana* promastigotes are capped by the trisaccharide Galβ1,4(Manα1,2)Manα1 [9, 12].

Lipophosphoglycan functions. Due to its unique carbohydrate component, lipophosphoglycan is able to fulfill a number of functions necessary for survival and propagation of *Leishmania* in sand-flies and humans.

1. *With the help of lipophosphoglycan, Leishmania joins sand-flies stomach epithelium cells.* A certain sand-fly species is able to transfer just one *Leishmania* species, and the lipophosphoglycan is the main molecule responsible for the interaction of the insect with the parasite. The carbohydrate part of the lipophosphoglycan serves as a ligand for lectins present on the stomach epithelium, binding to which is considered as the basis for attachment [17]. No binding takes place in the case of *Leishmania* entry into the stomach of incompatible sand-fly species because in this case there are no suitable receptors on the stomach epithelium, and the parasite is eliminated from the insect's body. An example of such interaction can be the PpGalec protein actively expressed in stomach of female imagoes of *Phlebotomus papatasi* and *Phlebotomus duboscqi*. PpGalec is a tandem type galectin with molecular mass 34.5 kDa and contains two Gal-binding domains separated by a linker. It binds lipophosphoglycan of *L. major* containing Galβ1,3 in side chains but does not recognize lipophosphoglycan of different *Leishmania* species such as *L. donovani* (side chain-free lipophosphoglycan) and *L. tropica* whose side chains carry glucose and arabinose [18]. Just *P. papatasi* and *P. duboscqi* are natural vectors of *L. major*, and they are not able to transfer other *Leishmania* species. Lipophosphoglycan also plays the leading role in the reverse process when it is necessary for *Leishmania* promastigotes to separate from stomach epithelium and enter the sand-fly salivary glands. To achieve this, lipophosphoglycan undergoes elongation, changes its spatial form and side chain composition, which results in masking epitopes involved in binding stomach receptors and parasite release [19].

2. *Lipophosphoglycan protects Leishmania cells against lysis by the complement system via hindering the assembly of C5b-9 membrane-attacking complex.* Long lipophosphoglycan chains of *L. major* form a protective barrier around the parasite cell and sterically prevent the attachment of C5b-9 complex to the promastigote membranes [20, 21]. *Leishmania donovani* escapes C5b-9 formation on its cell surface using an alternative mechanism in which lipophosphoglycan of *L. donovani* binds only inactive form of C3b (C3bi) without participation in C5 convertase formation [20]. The bound C3 protein is then removed from the parasite surface by proteolysis, probably carried out by a protein of the *L. donovani* cell surface.

3. *Lipophosphoglycan protects promastigotes against oxidative burst during phagocytosis. Leishmania major* mutants that do not synthesize lipophosphoglycan are able to infect *phox*-macrophages as efficiently as the wild-type *L. major* does. *Phox*-macrophages are free of phagocyte oxidase, the enzyme that synthesizes reactive oxygen forms toxic for microbes; therefore, experiments with them confirmed the protective role of lipophosphoglycan in phagocytosis [22]. This supposition agrees well with data showing that lipophosphoglycan shields against oxy-

gen radicals *in vitro* and that the lipophosphoglycan-free mutant of *L. major* is more susceptible to oxidative stress compared to the wild-type promastigotes [23].

4. *Lipophosphoglycan regulates NO synthase expression in macrophages.* Elimination of the pathogen by macrophages using nitric oxide (NO) is among the main protective mechanisms of the immune system. In response to extracellular signals like interferon-γ or bacterial lipopolysaccharide, NO synthase, which synthesizes the pathogen-toxic NO, is induced in macrophages. Lipophosphoglycan isolated from *Leishmania* promastigotes inhibited the NO synthase expression in mouse macrophage culture, thus significantly decreasing the antipathogenic function of these cells [24]. The phosphoglycan part of lipophosphoglycan molecule is responsible for this regulatory effect because significant decrease in synthesis of the NO-synthase mRNA, and respectively, of NO, was observed in macrophages incubated with artificially synthesized fragments of lipophosphoglycan repetitive units [24].

5. *Lipophosphoglycan retards maturation of macrophagic phagolysosomes.* Within macrophages, *Leishmania* promastigotes penetrate into the phagosome that should then form a mature phagolysosome by fusion with endosomes. Many experimental data show that lipophosphoglycan prevents the fusion of phagosomes with endosomes via retardation of phagolysosome formation [25]. For example, phagosomes containing promastigotes of the wild-type *L. donovani* or latex with immobilized lipophosphoglycan did not express late endocytic and lysosomal markers rab7 and LAMP1. In experiments with lipophosphoglycan-free *L. donovani* or with uncovered latex globules, phagosomes quickly acquired rab7 and LAMP1, and phagolysosome maturation proceeded at the normal rate [26-29]. Retarded formation of phagolysosomes is necessary for *Leishmania* so that the promastigotes have enough time for differentiation to amastigotes, and hydrolytic enzymes of mature phagolysosome could not destroy them.

6. *Lipophosphoglycan inhibits IL-12 secretion by macrophages.* Macrophages, initiated by *Leishmania*, did not secrete cytokine IL-12 necessary for development of protective $CD4^+$ T-cell immune response [30]. Inhibition of IL-12 secretion was also modulated by purified lipophosphoglycan [31-33], probably working as a specific gene-silencer with regard to the *IL1β* gene promoter [34].

Glycosylinositol phospholipids (GIPL) are the family of small glycolipids containing the Manα1,4GlcNα1,6-*myo*-inositol-1-phospholipid structure [14, 35, 36]. *Leishmania* actively express GIPL (approximately 10^7 molecules per cell) on the cell surface both at the promastigote and amastigote stages.

GIPL structure. Based on the structure of the glycan part, the following GIPL are distinguished: *type 1*, in which Manα1,6 Manα1,4GlcNα1,6-PI is the basis of the glycan part (similarly to protein GPI anchor); *type 2*, in which Manα1,3Manα1,4GlcNα1,6-PI is the basis of the glycan part (similarly to lipophosphoglycan anchor); *the hybrid type* in which the glycan part combines features of the first two types and is based on the branched structure Manα1,6(Manα1,3)Manα1,4GlcNα1,6-PI.

The three main types are in their turn separated into species depending on amount of mannose and galactose residues joined to the base; at the present time about twenty GIPL species are known. The GIPL lipid part is 1-*O*-alkyl-2-*O*-acylglycerol or *lyso*-1-*O*-acylglycerol; GIPL of some *Leishmania* species like *L. panamensis*, contain diacylglycerol lipid [37]. Aliphatic chains of type 1 GIPL (C18:0) are usually shorter than those in type 2 (C24:0 or C26:0).

Structural similarity to the protein and lipophosphoglycan GPI anchors suggests that GIPL are byproducts of biosynthesis of these macromolecules, but a high amount of GIPL on the membrane (1000-fold exceeding that of GPI anchored proteins) as well as different lipid components and modifications of the glycan part favor the existence of independent GIPL biosynthesis pathways [37]. These small glycolipids cover a significant part of *Leishmania* cells (30-100%) and at the stage of amastigote in the absence of lipophosphoglycan they remain the main molecules on the membrane [38].

Functions of GIPL. Despite high amount of GIPL on the *Leishmania* cell surface, very little is known about their functions. Structurally similar glycolipids are synthesized by all members of the Trypanosomatidae family, thus suggesting their importance for the protozoan cells [39]. Thus, synthesis of GIPL sharply increases in *Trypanosoma brucei* mutants that have lost their main cell surface glycoprotein [40]. Supposedly, GIPL play an important role in penetration of *Leishmania* promastigotes into macrophages, acting as ligands for the blood serum mannose-binding protein or macrophagic mannose-fucose receptor [41]. Since GIPL are prevalent on the amastigote cell surface, they might be involved in the protection of the parasite against destructive environment of macrophagic phagolysosomes. Some investigations have also shown that GIPL are able to influence the host immune response because purified GIPL modulated NO synthesis and oxidative burst in immune system cells [42-44]. It is especially intriguing that enzymes involved in GIPL biosynthesis are absolutely necessary for growth of *L. mexicana* promastigotes [45], which emphasizes the importance of GIPL for *Leishmania* cells.

GP63. The main protein of the cell surface of *Leishmania* promastigotes is GP63, a zinc proteinase with molecular mass 58-65 kDa, fixed at the membrane by the GPI anchor (Fig. 2). On average, 500,000 GP63 copies are expressed per cell, which makes up about 1% of the total cellular protein. *Leishmania* amastigotes decrease GP63 expression; in this case most of the protein loses the GPI anchor and appears in a flagellar pock-

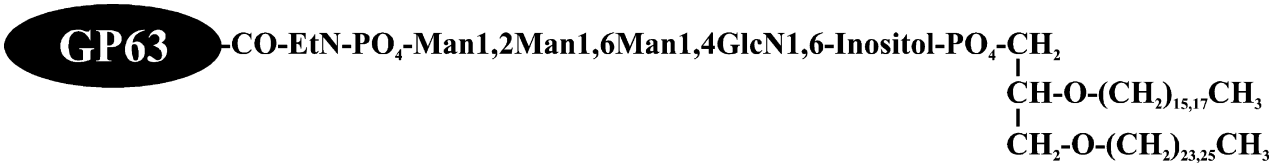


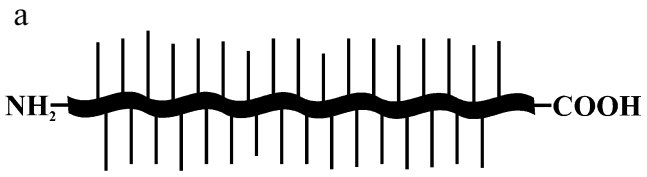
Fig. 2. Structure of GPI anchor of GP63.

et instead of the cell surface [46]. GP63 is encoded by a tandem of closely arranged genes (seven genes in *L. major*, 15 in *L. mexicana*, and 18 in *L. chagasi*), highly conserved in the whole *Leishmania* genus [47-49]. The main GP63 isoform expressed on the promastigote membrane is modified by three N-bound glycans that are double-antenna chains of six mannose residues usually terminated by glucose. At the stage of amastigotes the structure of this protein changes, the carbohydrate chain composition becomes more diverse, and GP63 of *L. donovani* amastigotes is not glycosylated on the whole [50, 51]. The role of structural alteration of GP63 glycans at different stages of *Leishmania* life cycle is still unknown.

GP63 appears to have the following functions: it hydrolyzes the opsonizing components of the complement system from the promastigote cell surface [52]; it serves as a ligand (direct or indirect, after complement activation) for macrophage receptors such as mannose-fucose receptor, receptor of complement 3 system, CD11b-CD18, and fibronectin receptor [52]; it hydrolyzes proteins of extracellular matrix [53]. The *L. major* mutant promastigotes devoid of GP63 are more sensitive to lysis by the complement system compared to wild-type promastigotes [54]. Simultaneously, amastigotes of mutants retain virulence identical to that of the wild type, thus supporting the supposition that GP63 is not necessary to maintain infection inside macrophages.

Proteophosphoglycans. The proteophosphoglycan family consists of secreted or membrane-anchored proteins strongly modified by phosphoglycan chains resembling repetitive phosphoglycan units [13, 15, 55] (Fig. 3). This type of protein modification, called phosphoglycosylation, is widespread in Protozoa of the Trypanosomatidae family and includes the [6Galβ1,4Manα1-PO_4]$_n$ fragment joining to serine-containing regions of proteophosphoglycan. The repertoire of expressed proteophosphoglycans depends on the *Leishmania* species and the stage of its life cycle [13, 15, 56]. For example, *L. mexicana* promastigotes secrete three different species: two forms of secreted alkaline phosphatases (sAP1,2) and filamentous proteophosphoglycan (fPPG), whereas amastigotes secrete only one species (aPPG) accumulated in large amounts in the parasitophore vacuole and intercellular space of human skin [57]. Compared to proteophosphoglycans of promastigotes, aPPG has an altered polypeptide chain and structurally more complex phosphoglycans [13, 15, 58]. Different *Leishmania* species secrete different amounts of proteophosphoglycans, and some strains of *L. major* [59] also contain on their cell surface GPI-anchored protein forms [60]. It is supposed that different proteophosphoglycan species fulfill different functions, but these are still poorly studied. In particular, fPPG is gathered in large macromolecular complexes and forms a viscous net around the parasite cells, thus protecting them against proteases present in mosquito stomach [17]. fPPG is also necessary for *Leishmania* survival at early stages of infection, supposedly because it protects promastigotes against the blood-stream hydrolytic environment [17]. In macrophages, secreted aPPG are involved in formation of the parasitophore vacuole [61] and, due to high mannose content in glycan chains, in complement system activation via mannose-binding receptor [62, 63].

Biosynthesis of *Leishmania* phosphoglycans. GPI anchor biosynthesis. *Leishmania* synthesize phosphoglycans by consecutive joining of monosaccharide to phosphatidylinositol (PI) in the endoplasmic reticulum and Golgi apparatus. A scheme of *Leishmania* phosphoglycan biosynthesis is shown in Fig. 4. Only 1% of PI molecules having long (C24:0/C26:0) alkyl chains are involved in formation of protein GPI anchors, while a much higher amount of phosphatidylinositol (about 20% of the total pool) with short (C18:0) chains are used for GIPL synthesis [64]. Such selectivity can be achieved due to specificity of one or several enzymes for a certain lipid sub-



b

Manα1,2Manα1-P-Ser

Araβ1,2Galβ1,3[Galβ1,4Manα1-P]$_n$Galβ1,4Manα1-P-Ser

Fig. 3. Proteophosphoglycan of *Leishmania*. a) Peptide chain (wavy line) highly glycosylated by phosphoglycans (straight lines). b) Structure of carbohydrate chains of *Leishmania* proteophosphoglycan with phosphoglycosylation at serine residues.

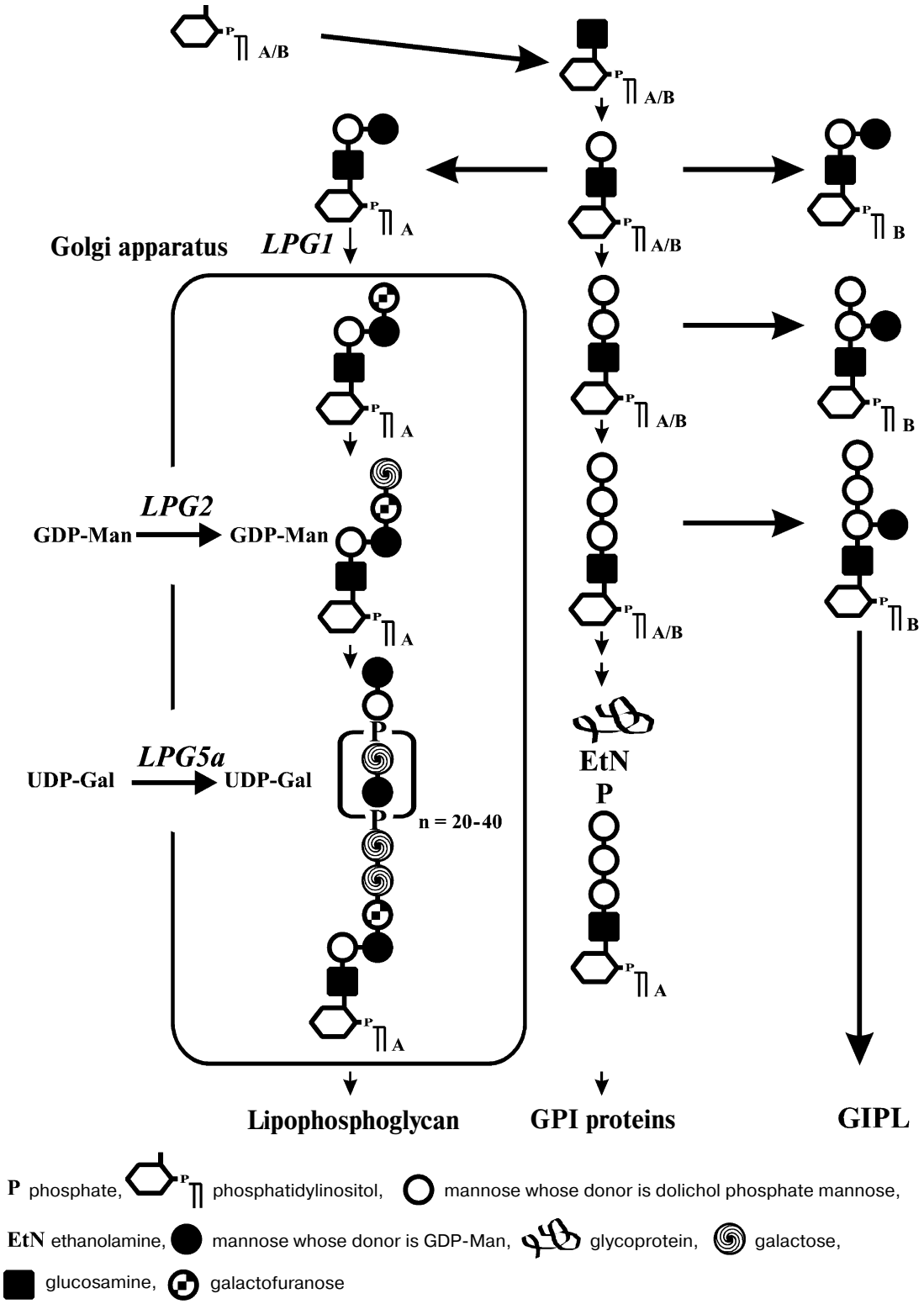


Fig. 4. Biosynthesis of *Leishmania* phosphoglycans (adapted from [80] and supplemented by recent data of N. M. Novozhilova). GPI anchor and phosphoglycolipids are synthesized by consecutive joining of monosaccharides to phosphatidylinositol with long C24:0/C26:0 (A) or short C18:0 (B) alkyl chains. Carbohydrate parts of phosphoglycans of all types contain similar regions. GPI anchor and GIPL are synthesized in endoplasmic reticulum, while lipophosphoglycan is synthesized in Golgi apparatus. Some biosynthetic reactions such as deacylation of *N*-acetylglucosamine, ethanolamine-phosphate joining to GPI anchor, and remodeling of fatty acids are not shown to simplify the scheme. Some presently known genes involved in lipophosphoglycan biosynthesis are shown.

strate during biosynthesis of molecules of different classes. According to another hypothesis, enzymes of different biosynthetic pathways are localized in separate subcompartments of endoplasmic reticulum, each of which is saturated by only a single type of lipids.

Based on data on biosynthesis of a similar GPI anchor of *Trypanosoma brucei* [65], one can suppose that the carbohydrate part of all three classes of *Leishmania* phosphoglycans (lipophosphoglycan, GIPL, and protein GPI anchor) is synthesized via the common precursor Manα1,4GlcN-phosphatidylinositol. In *T. brucei* Manα1,4, the first mannose of the glycan part, is joined by the corresponding α1,4-mannosyl transferase in endoplasmic reticulum. Ferguson et al. [65] tried to study this enzyme and developed a method for testing its substrate specificity, but they stopped further investigations because the isolated proteins were unstable. Mannose donor for *T. brucei* α1,4-mannosyl transferase is dolichol phosphate-mannose synthesized from GDP-Man and dolichol phosphate using dolichol phosphate-mannose synthase (DPMS) [66]. Attempt to knock out a similar *DPMS* gene in *L. mexicana* resulted in death of the *Leishmania* cells, thus confirming that synthesis of GPI-containing molecules is vital for the protozoon [45]. The protein GPI anchor of *T. brucei* is formed in endoplasmic reticulum by addition to Manα1,4GlcN-PI of two mannose molecules (Manα6 and Manα2, the donor for which is also dolichol phosphate-mannose [66]) and ethanolamine-phosphate.

Biosynthesis of the GPI anchor and lipophosphoglycan carbohydrate part is branched at the stage of the glycan core second mannose joining: in precursors of the first it joins with formation of an α1,4 bond, while in the case of the second an α1,3 bond is formed. In lipophosphoglycan the Manα3 is joined in the endoplasmic reticulum [67], and total further synthesis proceeds in the Golgi apparatus [68]. The third residue in lipophosphoglycan core is galactose in the form of furanose, which is not typical of eukaryotes. It is transferred by the corresponding transferase localized in the Golgi apparatus and representing type II transmembrane protein encoded by the *LPG1* gene [69]. *LPG1* knockout selectively stops lipophosphoglycan biosynthesis but does not affect syntheses of GPI anchors, proteophosphoglycans, and GIPL [23].

Biosynthesis of lipophosphoglycan repetitive units. The region of repetitive units is the most characteristic feature of lipophosphoglycans. Its assembly in Golgi apparatus requires participation of GDP-Man and UDP-Gal transporting proteins, three mannosyl-phosphate transferases exhibiting different substrate specificities, as well as β1,4-galactosyl transferase. The Gal and Man donors for this biosynthesis are UDP-Gal and GDP-Man synthesized in the cytoplasm and transferred into Golgi apparatus via membrane transporters. GDP-Man is delivered into Golgi apparatus by a multispecific protein encoded by the *LPG2* gene, which, along with GDP-Man, is also able to transfer GDP-Ara and GDP-Fuc [70]. The *lpg2*(−) mutants of *L. major* are completely devoid of lipophosphoglycan and carbohydrate chains of proteophosphoglycan synthesized in Golgi apparatus [23]. A minimum two proteins, LPG5A and LPG5B, involved in biosynthesis of various glycoconjugates, are responsible for UDP-Gal transport within the Golgi apparatus [71]. Synthesis of phosphoglycans was completely stopped only in cells of *L. major* double mutant *lpg5a*(−)/*5b*(−); switching off the *LPG5a* gene resulted in inhibition of lipophosphoglycan biosynthesis, while *LPG5b* knockout resulted in changes in synthesis of proteophosphoglycan carbohydrate chains. The mechanism providing for such unusual functional differentiation of transporting proteins with equal substrate specificity is still unknown [71].

Inside Golgi apparatus, GDP-Man is used by mannosyl-phosphate transferases joining mannose-1-phosphate to growing carbohydrate chains. *Leishmania* phosphoglycans have three different acceptor sites for joining Manα1-PO_4; these are Galα1,6Galα on the glycan core and Galβ1,4Manα1-PO_4 on the lipophosphoglycan repetitive units, as well as serine in the case of proteophosphoglycans.

Synthesis of lipophosphoglycan repetitive units begins from joining of Manα1-PO_4 mannosyl-phosphate at Galα1,6Gal of the glycan core. The corresponding mannosyl-phosphate transferase was isolated from microsomes of *L. donovani* [72], but the enzyme was poorly studied. After joining the first unit, following repetitive units are synthesized by a different elongating mannosyl-phosphate transferase requiring the presence of Manα1-PO_4 on the acceptor [73, 74]. Ferguson et al. [74] characterized in detail elongating mannosyl-phosphate transferases of promastigotes of three *Leishmania* species: *L. donovani*, *L. major*, and *L. mexicana*. Using synthetic analogs of lipophosphoglycan fragments, they showed that the phosphodiester negative charge and C6 hydroxyl of αMan are necessary for substrate recognition by the enzyme, while C6 hydroxyl of αMan probably also plays the role of the hydrogen bond acceptor. The C6 hydroxyl of βGal is also involved in substrate recognition and catalysis of the enzymatic reaction. The presence of monosaccharide substituent in C3 position of terminal βGal stopped the joining of Manα1-PO_4, thus indicating that synthesis of lipophosphoglycan repetitive units comes first and then modification by their side substituents follows [74]. The repetitive unit synthesis is completed by galactose transfer from UDP-Gal to Manα1-PO_4 catalyzed by β1,4-galactosyl transferase [73].

Lipophosphoglycan side chains. Many *Leishmania* species modify lipophosphoglycan side chains consisting mainly of β-D-Gal residues. For example, *L. major* promastigotes intensively galactosylate repetitive units by Galβ1,3 short chains necessary for the parasite to join the

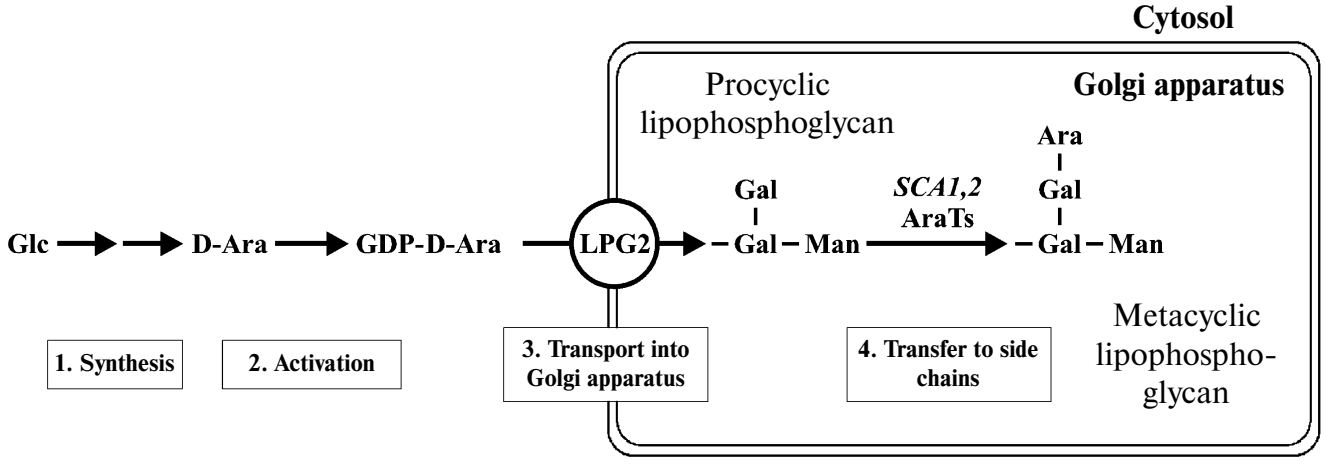
sand-fly stomach cells. A family of six genes encoding the supposed galactosyl transferases was found in the *L. major* genome. At least four proteins exhibited significant β-galactosyl transferase activity towards lipophosphoglycan side chains, one of which, SCG3, exhibited initiating activity, while another, SCG2, exhibited initiating and elongating β-galactosyl transferase activities [75].

At certain stages of the life cycle of *L. major*, it synthesizes lipophosphoglycan whose side chains are terminated by rare β-D-arabinopyranose. This modification is provided by two arabinopyranosyl transferases of side chains SCA1 and SCA2 [75].

GIPL biosynthesis. As mentioned above, GIPL can contain glycan cores analogous to protein and lipophosphoglycan anchors. Therefore, it is highly probable that they are synthesized by the same or very similar glycosyl transferases. On the other side, the GIPL lipid part differs greatly from other *Leishmania* phosphoglycans, while glycan core can contain additional modifications absent from lipophosphoglycan and GPI anchor [54, 64]. It is still not clear whether GIPL are products of a separate biosynthetic pathway or if they are formed as "excesses" of synthesis of lipophosphoglycan and protein GPI anchors. Most accumulated data support the first hypothesis. First, GIPL are the main molecules of the amastigote cell surface, while biosynthesis of other phosphoglycans significantly decreases [38]. Second, knockout of the *L. major* gene *LPG1*, encoding galactofuranosyl transferase, joining the fourth monosaccharide to glycan core, stops only lipophosphoglycan synthesis. *Leishmania major lpg1*(−) continues GIPL synthesis, although some GIPL species structurally resemble lipophosphoglycan and also contain galactofuranose in glycan core [76]. Thus, it is highly probable that *Leishmania* express two galactofuranosyl transferases, one of which is involved in biosynthesis of lipophosphoglycan, while the other takes part in biosynthesis of glycosylinositol phospholipids.

Arabinose-containing glycoconjugates of *Leishmania*. Arabinose is incorporated in many cell surface molecules of bacteria, protozoa, and plants, but it is absent from mammals. In natural compounds it exists mainly in the form of L-arabinofuranose, while D-arabinopyranose is extremely rare. At certain stages of their life cycle, *Leishmania* incorporate D-arabinopyranose into lipophosphoglycan, which is important for the survival of the parasite in the mosquito. In the case of entry with blood into the insect alimentary canal, *L. major* is attached to stomach epithelium due to recognition of the lipophosphoglycan side chains, β1,3Gal, by galectin PpGalec [18]. Subsequently *L. major* synthesizes lipophosphoglycan whose side chains are terminated by β1,2-D-arabinopyranose, and PpGalec stops galactose recognition within D-Araβ1,2[Galβ1,3]$_{1\text{-}3}$, thus allowing the parasite to separate from stomach epithelium and migrate into the insect's salivary glands, from which it gets into the human blood stream.

Biosynthetic pathways with the participation of D-arabinopyranose are practically unstudied, and since this monosaccharide is absent from mammalian cells, their understanding might reveal potential targets for design of drugs against leishmaniasis. Scheme summarizes the presently available data on D-arabinopyranose biosynthesis in *Leishmania*. In particular, *L. major* is able to synthesize D-arabinose from D-glucose by the cleavage of the C1 fragment from Glc [77], although the corresponding biosynthetic enzymes still remain unstudied. *Leishmania* are also able to absorb D-Ara from the environment. The D-arabinopyranose donor for biosynthesis of *Leishmania* and *Crithidia* glycoconjugates is GDP-D-Ara [78]. A possible route of GDP-D-Ara synthesis is phosphorylation of arabinose by arabinose-1-kinase with formation of arabinose-1-phosphate and then of GDP-D-Ara. Among enzymes involved in the supposed biosynthetic pathway, only D-arabino-1-kinase of the parasitic protozoon *Crithidia fasciculata* has been isolated and partially characterized [78]. The 600 kDa protein or protein complex of *C. fasciculata* also exhibited L-fucose-1-kinase activity, which is not surprising because L-fucose and D-arabinose are structurally similar and differ only by the presence of an additional methyl group in the



fucose C5 position. GDP-D-Ara-pyrophosphorylase is also necessary for the supposed biosynthetic pathway, but this enzyme has not yet been found. Nothing is known about enzymes involved in GDP-D-Ara biosynthesis in *Leishmania*. By analogy with other activated carbohydrates, the GDP-D-Ara synthesis should take place in the cytoplasm, which makes necessary GDP-D-Ara transfer into the Golgi apparatus. In *Leishmania* this can occur using the multispecific transporter protein LPG2 exhibiting activities towards GDP-Man, GDP-D-Ara, and GDP-L-Fuc. It is not known whether there are different GDP-D-Ara transporters in *Leishmania* besides LPG2. After entering the Golgi apparatus, GDP-D-Ara is used by D-arabinopyranosyl transferases SCA1 and SCA2 for lipophosphoglycan arabinosylation [79].

During evolution, parasitic Protozoa including *Leishmania* developed mechanisms allowing them to survive in extremely unfavorable conditions. In this connection members of the *Leishmania* genus are of special interest because they are able to affect macrophages designed for killing pathogens. The cell surface glycoconjugates play the most important role in the life of *Leishmania*; they mediate the interactions of the parasitic organism with vectors and hosts. Thick glycocalix protects the parasite against an extremely unfavorable environment and allows them to deceive human immune system, making possible infection and development. Owing to this, investigation of structure and biosynthetic pathways of phosphoglycans composing glycocalix of *Leishmania* extends our notion of the mechanism of action of this very efficient protective barrier and perhaps in future it will make it possible to reveal potential targets to design anti-leishmaniasis drugs.

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