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Phil. Trans. R. Soc. Lond. B 1997 **352**, 1295-1302
doi: 10.1098/rstb.1997.0113

The surface glycoconjugates of trypanosomatid parasites

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SUMMARY

Insect-transmitted protozoan parasites of the order Kinetoplastida, suborder Trypanosomatina, include *Trypanosoma brucei* (aetiological agent of African sleeping sickness), *Trypanosoma cruzi* (aetiological agent of Chagas' disease in South and Central America) and *Leishmania* spp. (aetiological agents of a variety of diseases throughout the tropics and sub-tropics). The structures of the most abundant cell-surface molecules of these organisms is reviewed and correlated with the different modes of parasitism of the three groups of parasites. The major surface molecules are all glycosylphosphatidylinositol (GPI)-anchored glycoproteins, such as the variant surface glycoproteins of *T. brucei* and the surface mucins of *T. cruzi*, or complex glycophospholipids, such as the lipophosphoglycans and glycoinositolphospholipids of the leishmanias. Significantly, all of the aforementioned structures share a motif of Manα1-4GlcNα1-6-*myo*-inositol-1-HPO$_4$-lipid and can therefore be considered to be members of a GPI superfamily.

1. INTRODUCTION

In this article, the most abundant surface molecules (more than 100 000 copies per cell) of three trypanosomatid parasites, *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania* spp., will be described and correlated with their modes of parasitism. These surface molecules are glycosylphosphatidylinosotil (GPI)-anchored glycoproteins and GPI-related complex glycophospholipids. Many other interesting and important surface molecules are found in these parasites but these will not be discussed here because their lower abundance means that they contribute less to the general surface architecture of the parasites.

2. THE SURFACE OF *TRYPANOSOMA BRUCEI*

The bloodstream forms of the African trypanosomes express an unusual surface coat (Vickerman & Luckins 1969) composed of about 10^7 copies of variant surface glycoprotein (VSG) (Cross 1975) arranged as a dense monolayer of homodimers on the parasite surface. The VSG coat acts as a macromolecular diffusion barrier that prevents the approach of macromolecules, such as the components of the alternative complement pathway, to the plasma membrane while allowing the free diffusion of small nutrient molecules to underlying transmembrane transporter systems. The trypanosome genome contains several hundred VSG genes that encode immunologically distinct VSGs that are expressed one at a time by an individual cell. The sequential expression of different VSG genes (antigenic variation) enables the parasite population to evade specific humoral immune attack; the mechanism of antigenic variation has been reviewed recently (Cross 1996).

Each VSG monomer has a molecular mass of approximately 55 kDa and contains typically one or two N-linked oligosaccharides (Zamze *et al*. 1991). The amino-terminal domains of the VSGs represent about 75% of the mature polypeptide sequences and show little primary sequence homology, thereby allowing the VSGs their unique immunological identities. However, X-ray crystallographic studies indicate that they fold into similar elongated shapes, with two extended α-helical bundles per monomer (Blum *et al*. 1993), that pack into dense monolayer coat arrays. This arrangement allows the coat to be quite thick (15 nm) and means that only a limited subset of VSG B-cell-stimulating epitopes are exposed on the surface of the living trypanosome. The carboxy-terminal domains of the VSGs display some sequence homology and the VSGs can be grouped into two main subtypes based on these sequences; however, the three-dimensional structures of these domains have not been solved. The VSG molecules are finally attached to the plasma membrane through a covalent linkage from the carboxy-terminal amino acid α-carboxy group to a GPI membrane anchor (figure 1*a*) (Ferguson *et al*. 1988). The mature VSG GPI anchors contain the core structure of ethanolamine-HPO$_4$-6Manα1-2Manα1-6Manα1-4GlcNα1-6PI, common to all GPI membrane anchors, but this is modified by an α-galactose side chain that is unique to VSGs. The predicted three-dimensional structure of the complete anchor suggests that it forms a large, dense plate-like structure of carbohydrate on which the VSG polypeptide sits (Homans *et al*. 1989). Thus the VSG coat can be considered as a dense GPI glycocalyx immediately adjacent to the membrane covered with the proteinacious part of the VSG layer (see figure 2).

The surface of the procyclic form of *T. brucei*, which lives in the tsetse fly midgut, is covered with a more

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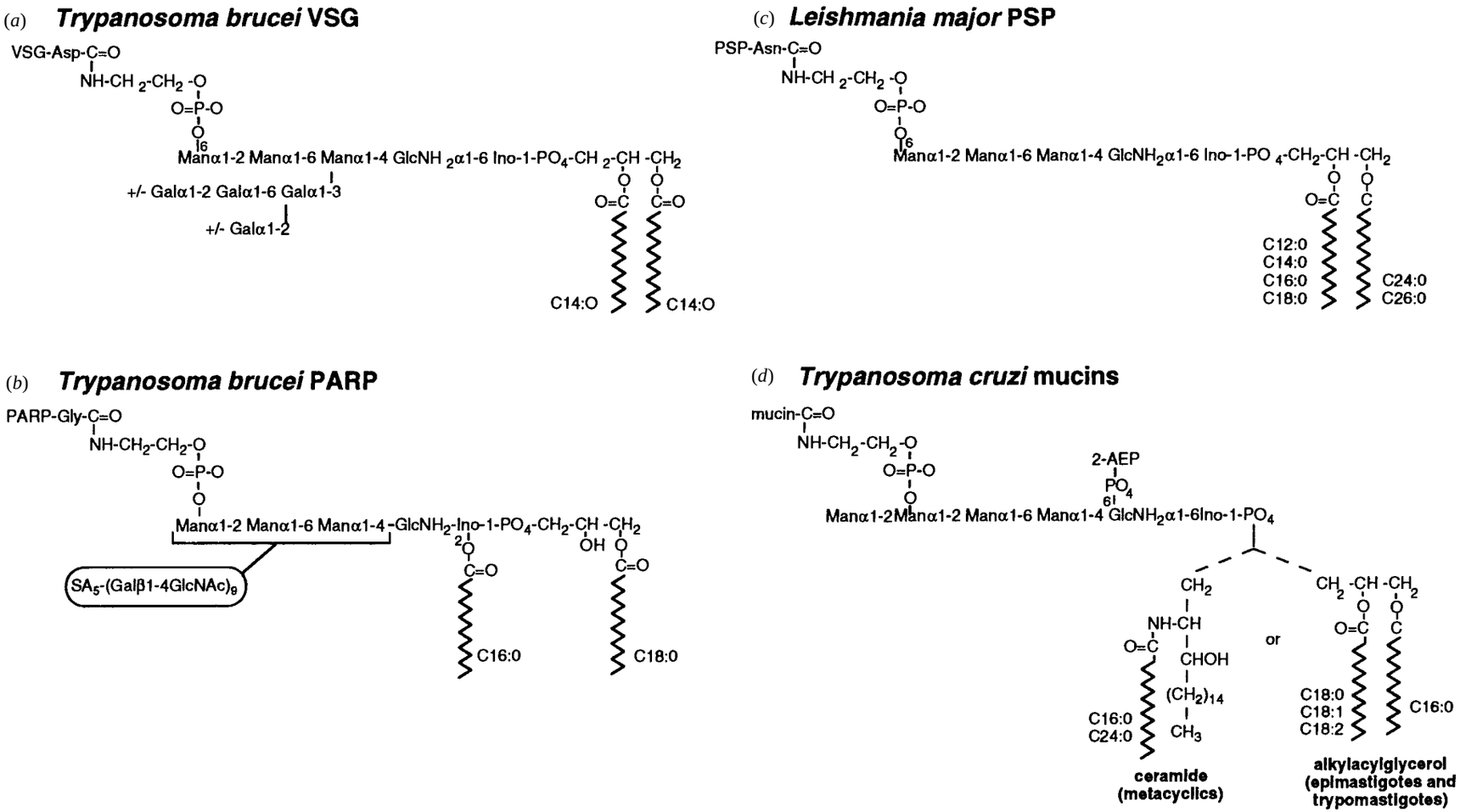

Figure 1. The structures of some trypanosomatid GPI-protein anchors. The structures of the GPI anchors of (*a*) *T. brucei* bloodstream-form VSG, (*b*) *T. brucei* procyclic PARP, (*c*) *L. major* PSP, and (*d*) *T. cruzi* metacyclic and epimastigote mucins are shown.

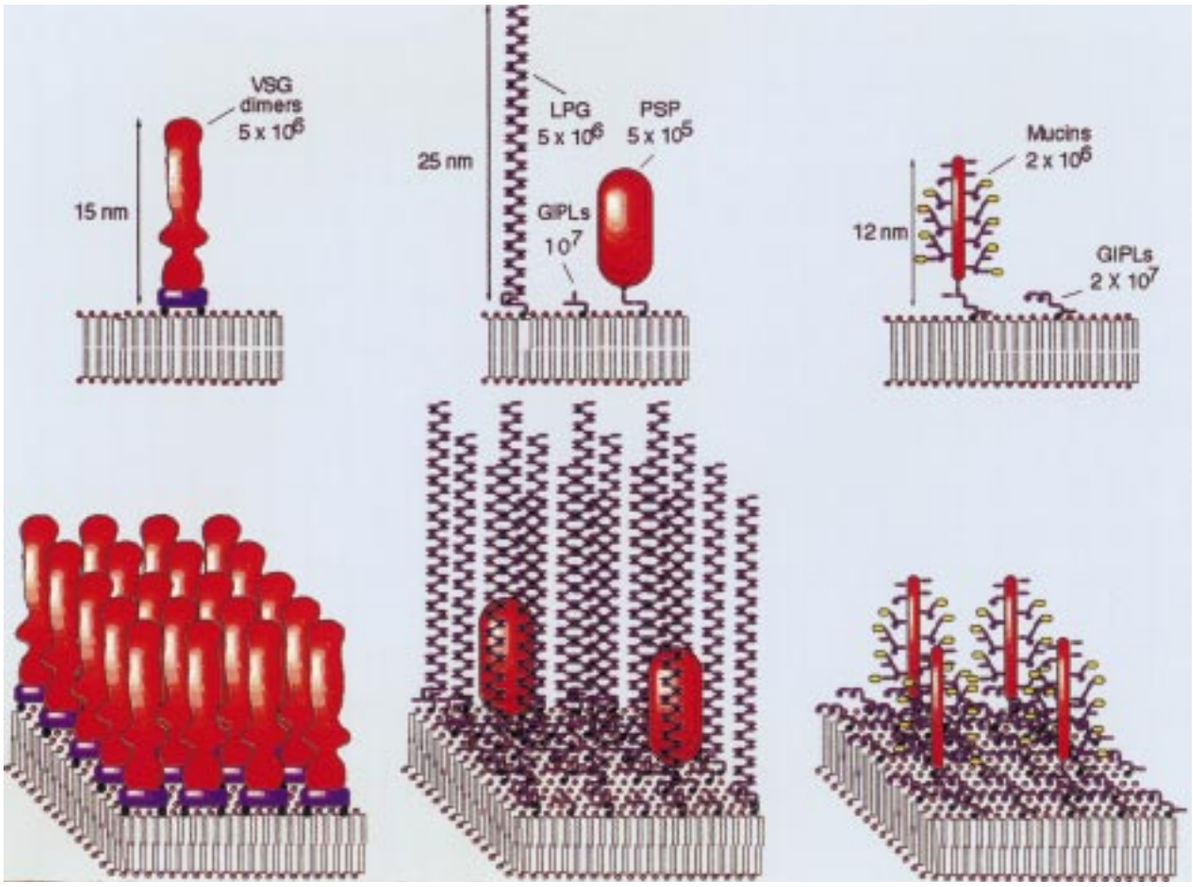

Figure 2. A representation of the cell surface molecular architecture of three trypanosomatid parasites. Schematic representations of the major surface molecules and a 20 nm × 20 nm section of plasma membrane of (left) a *T. brucei* bloodstream-form trypomastigote, (middle) a *Leishmania* metacyclic promastigote and (right) a *T. cruzi* metacyclic trypomastigote.

diffuse coat made up of glycoproteins called procyclins or procyclic acidic repetitive proteins (PARPs). Most trypanosome clones contain four pairs of PARP genes that encode three PARP types: two with repeat domains of -Glu-Pro- (EP-PARPs), one of which contains an N-glycosylation site adjacent to the polyanionic EP-repeat domain, and one with a repeat domain of -Gly-Pro-Glu-Glu-Thr- (GPEET-PARP) (Roditi *et al*. 1987; Mowatt & Clayton 1988). Both the N-glycoslyated EP-PARP species and the GPEET-PARP species have been isolated and structurally characterized. Both forms are believed to adopt highly extended rod-like structures (Roditi *et al* 1989; Treumann *et al*. 1997) and both contain GPI anchors with an unusual lipid structure and large polydisperse carbohydrate side chains made of branched polylactosamine repeats, i.e. Galβ1-4GlcNAc repeat units (figure 1*b*) (Field *et al*. 1991; Ferguson *et al*. 1993; Treumann *et al*. 1997). When the parasites are grown in culture, the β-galactose termini of these side chains are substituted with sialic acid through the action of a cell surface trans-sialidase enzyme that transfers α2–3-linked sialic acid from fetal calf serum glycoconjugates (Engstler *et al*. 1993). The significance of trans-sialylation in *T. brucei* procyclics, and the extent to which it occurs in the tsetse fly, are not clear. Overall, the PARP coat can be considered as a dense GPI glycocalyx immediately adjacent to the membrane above which are projected the highly extended polyanionic rod-like structures of the PARP polypeptides. The significance of the three different PARP forms, described above, is unclear. However, when bloodstream-form trypanosomes transform into procyclics *in vitro* the cells generally express predominantly N-glycosylated EP-PARP, which is gradually replaced by GPEET-PARP over a period of months (Treumann *et al*. 1997). It will be interesting to analyse which forms of PARP are being expressed by trypanosomes during their complex life-cycle within the tsetse fly. Recently, tsetse fly lectins have been suggested to be involved in controlling the parasite population in the tsetse fly (Maudlin & Welburn 1994; Welburn *et al*. 1996) and it is likely that the PARPs serve as the principal ligands for these molecules.

3. THE SURFACE OF THE *LEISHMANIA* SPP.

The most abundant cell-surface molecules of the promastigote stage of the *Leishmania* are a GPI-anchored glycoprotein called promastigote surface protease (PSP), a family of glycoinositol-phospholipids (GIPLs) and lipophosphoglycan (LPG) (McConville & Ferguson 1993). The PSP GPI anchor is a very simple one (figure 1*c*) and the GIPLs fall into three types according to their glycan structure (McConville

et al. 1993; McConville & Ferguson 1993). The Type-1 GIPLs are more similar to the GPI protein anchors and contain a core of Manα1-6Manα1-4GlcNα1-6PI, the Type-2 GIPLs contain a core of Manα1-3Manα1-4GlcNα1-6PI, and the hybrid-GIPLs contain a branched core of Manα1-3(Manα1-6)Manα1-4GlcNα1-6PI. Representative examples of the *Leishmania* GIPLs are shown in figure 3. The relative proportions of the different GIPL species varies between *Leishmania* species and between the promastigote and amastigote forms of the parasite (McConville *et al*. 1990; McConville & Blackwell 1991; McConville & Ferguson 1993; Schneider *et al*. 1993, 1994; Winter *et al*. 1994). The LPGs consist of a Type-2 GIPL membrane anchor attached to a long phosphosaccharide-repeat domain that is capped by a neutral oligosaccharide (McConville & Ferguson 1993; McConville *et al*. 1995) (figure 3). The *Leishmania* promastigote surface therefore consists of a dense GIPL glycocalyx immediately adjacent to the membrane through which the PSP and polyanionic LPG molecules are projected (figure 2).

The physicochemical similarity between the *Leishmania* cell surface and the African trypanosome procyclic cell surface is quite striking in that both surfaces have dense layers of carbohydrate immediately adjacent to the membrane with polyanionic rods displayed above. In the trypanosomes this is achieved with a single molecule (PARP) that supplies the glycocalyx in the form of its highly modified GPI anchor and the polyanionic rods in the form of extended acidic polypeptide repeats, whereas the *Leishmania* use GIPLs to form the glycocalyx and separate LPG molecules to supply the polyanionic rods in the form of sugar-phosphate repeats.

With the exception of *L. major* (Moody *et al*. 1993), the intracellular amastigote stages expresses little or no LPG and no major cell-surface glycoproteins (McConville & Blackwell 1991; Bahr *et al*. 1993). The phosphosaccharide-repeat domains of the LPGs carry side chain modifications that are species-specific (figure 3) (McConville *et al*. 1995). These differences in LPG structure seem to have a key role in defining the sandfly vector range of these organisms, suggesting that LPGs mediate parasite adhesion to putative sandfly midgut epithelium lectins (Pimenta *et al*. 1992, 1994). In *L.major* and *L. donovani*, differentiation of the non-infectious dividing procyclic promastigotes to infectious non-dividing metacyclic promastigotes is correlated with an approximate doubling in the length of the phosphosaccharide repeat domains and (for *L. major*) a change in the phosphosaccharide side chains (McConville *et al*. 1992; Sacks *et al*. 1995). These changes in LPG structure are responsible for the detachment of the parasites from the vector midgut epithelium and for their acquisition of resistance to complement. Interestingly, the metacyclic promastigote LPG is a potent activator of the classical complement pathway, and the opsonization of the metacyclic promastigote LPG layer with C3b and C3bi is essential for the successful invasion of host macrophages via the macrophage CR1/CR3 complement receptor system, reviewed in Brittingham *et al*. (1996). The resistance to complement-mediated lysis is thought to be due (*a*) to the length of the LPG molecule, which holds the complement cascade far enough away from the parasite membrane to prevent insertion of the membrane attack complex, and (*b*) to the action of the PSP protease that can degrade C3b to C3bi-like products. The activation of complement by the classical pathway is thought to be due to the nature of the LPG cap structure (which contains α-mannose residues) that efficiently binds serum mannose-binding protein (MBP), which has a complement-activating Clq-like domain (Green *et al*. 1994). The LPG has also been implicated in inhibiting the macrophage oxidative burst, providing further protection for the parasite once inside the macrophage (Turco & Descoteaux 1992). Recently, the GIPLs and LPG (and synthetic LPG fragments) have been implicated in modulating the macrophage inducible nitric oxide synthase (iNOS) (Proudfoot *et al*. 1995, 1996) and these features are reviewed elsewhere in this volume by Liew *et al*.

The novel phosphosaccharide repeats found in the LPGs are also found attached to protein as part of the so-called proteophosphoglycans that are secreted by *Leishmania* promastigotes and amastigotes as large macromolecular filaments and networks (Ilg *et al*. 1994*a*,*b*, 1996). These large molecules might have an important role in protecting the amastigote parasite inside the macrophage (Ilg *et al*. 1996).

4. THE SURFACE OF *TRYPANOSOMA CRUZI*

The most abundant cell-surface molecules on the epimastigote and metacyclic forms of this parasite are a small family of Type-1 GIPLs (figure 3) (Previato *et al*. 1990; Lederkremer *et al*. 1991, 1993; Carreira *et al*. 1996) and a family of GPI-anchored heavily O-glycosylated mucin-like glycoproteins (Schenkman *et al*. 1993; Previato *et al*. 1994, 1995; Serrano *et al*. 1995). The *T. cruzi* GIPLs are predicted to form a dense glycocalyx immediately adjacent to the membrane, with the mucins projecting out above this layer (figure 2). The GPI anchors of the mucins are relatively simple (figure 1*d*) but there is an interesting change in their lipid structure when the epimastigotes differentiate into metacyclic trypomastigotes. The epimastigote mucin GPI anchors contain exclusively *sn*-1-alkyl-2-acylglycerol, whereas over 70% of the metacyclic mucin GPI anchors contain ceramide lipids (Serrano *et al*. 1995). The significance of this change in lipid structure is unclear but it might correlate with the ability of the mucins to insert into the host cell membrane during invasion, whereas another metacyclic GPI-anchored glycoprotein (the 1G7 antigen), which contains predominantly *sn*-1-alkyl-2-acylglycerol (Heise *et al*. 1995), remains attached to the parasite (Schenkman *et al*. 1993).

The bloodstream forms of *T. cruzi* also express abundant GPI-anchored mucins, although these seem to be larger than the epimastigote/metacyclic mucins (Almeida *et al*. 1994), and the GPI anchor lipid component in this case is *sn*-1-alkyl-2-acylglycerol. The acyl (fatty acid) chains of the bloodstream trypomastigote GPI lipid are mostly unsaturated fatty acids ($C_{18:1}$ and $C_{18:2}$), whereas the *sn*-1-alkyl-2-acylglycerol lipids from the other life-cycle stages contain exclusively saturated

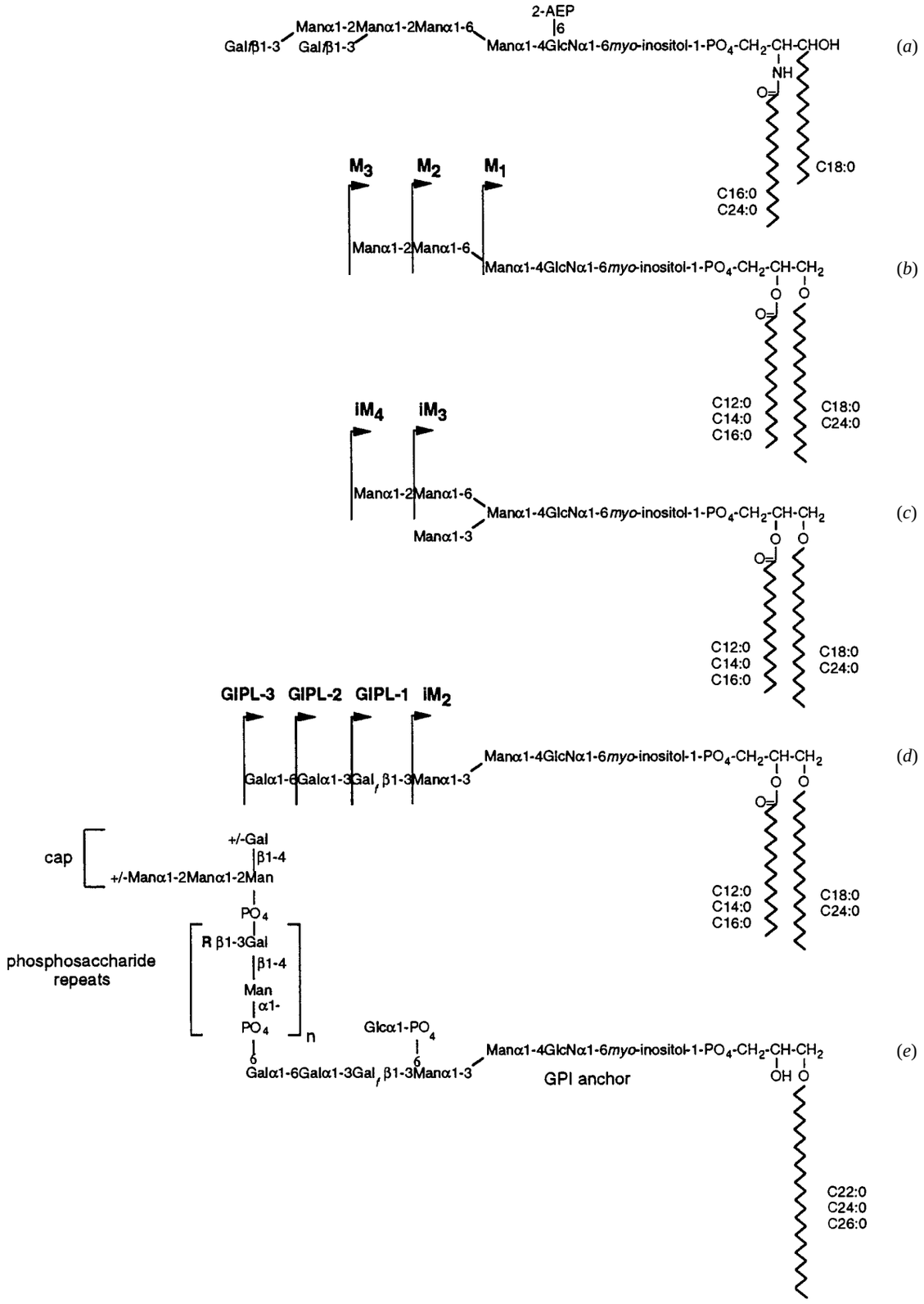

Figure 3. 1 Representative examples of the Type-1 and Type-2 GIPLs and LPGs. The arrows on the *Leishmania* GIPLs indicate the names of the smaller GIPL species (McConville & Ferguson 1993). The structure of the side chains (R) and the average number of phosphosaccharide repeats (*n*) of the *Leishmania* LPGs varies depending on the species and developmental stage of the parasite (McConville & Ferguson 1993; McConville *et al*. 1995). (*a*) *T. cruzi* Type-1 GIPL, (*b*) *Leishmania* Type-1 GIPLs, (*c*) *Leishmania* hybrid GIPLs, (*d*) *Leishmania* Type-2 GIPLs, and (*e*) *Leishmania* LPGs.

fatty acids. This subtle difference in lipid structure might be immunologically significant because the bloodstream trypomastigote mucins, but not the epimastigote and metacyclic mucins, are potent inducers of host macrophage tumour necrosis factor α and IL-12 (Carmago *et al*. 1997). The mucin O-linked oligosaccharides seem to play an important role in *T. cruzi* metacyclic and bloodstream trypomastigote infectivity. The mucins are the major acceptor substrates for the cell surface trans-sialidase enzymes of the parasite, which transfer α2-3-linked sialic acid from host glycoconjugates to the parasite surface (Schenkman *et al*. 1994). Trans-sialyation seems to be necessary for host cell invasion (Schenkman *et al*. 1991), and the O-linked oligosaccharides that terminate in β-galactopyranose residues (e.g. Gal*p*β1-3(Gal*p*β1-6)Gal*p*β1-6(Gal*f*β1-4) GlcNAO-Thr) are the specific sialic acid acceptor sites (Previato *et al*. 1994, 1995; Serrano *et al*. 1995). The available structural data show that the O-linked oligosaccharides of the epimastigote mucins show some variation between *T. cruzi* strains (Previato *et al*. 1994, 1995) but that within a given strain the epimastigote and metacyclic structures are identical (Serrano *et al*. 1995). In contrast, the bloodstream trypomastigote mucin oligosaccharides uniquely contain α-galactose residues that are responsible for eliciting the high-levels of anti-αGal antibodies seen in patients with Chagas' disease (Almedia *et al*. 1994). Several *T. cruzi* mucin genes belonging to a multi-gene family have been cloned (Noia *et al*. 1995; Salazar *et al*. 1996) but it is not yet clear which of them are expressed in the various life-cycle stages.

Although a small number of *Leishmania* amastigote surface glycoproteins have been identified (Andrews *et al*. 1988; Teixeira *et al*. 1994), relatively little is known about the surface architecture of this stage of the parasite.

5. OTHER TRYPANOSOMATID PARASITES AND GIPL PHYLOGENY

A number of other trypanosomatid parasites have been analysed for glycolipid/glycoconjugate content and structure. In all cases, the major molecular species belong to the GPI family; for example, Type-1 GIPLs have been described in *Phytomonas* (Redman *et al*. 1995) and Type-2 and/or hybrid GIPLs have been described in *Leptomonas samueli* (Previato *et al*. 1992), *Endotrypanum schaudinni* and *Herpetomonas samuelpessoai* (Routier *et al*. 1995), and *Crithidia fasciculata* (Schneider *et al*. 1996). A wide variety of glycosyl groups are found attached to the aforementioned GIPLs, including αMan, αGal*p*, βGal*f*, αGlc, βXyl, αRha, αGlcA and D-Ara*p* residues, and a number of phosphorus-containing substituents are also found, including ethanolamine phosphate, 2-aminoethylphosphonate and Glcα1-HPO$_4$-. A recent analysis of trypanosomatid small-subunit rRNA sequences (Fernandes *et al*. 1993) suggests that: (i) *T. cruzi* is more closely related to *Phytomonas* than to *Leptomonas*, *Endotrypanum* and *Leishmania*, (ii) *Leptomonas*, *Endotrypanum* and *Leishmania* are more closely related to each other than to *Photomonas* and *T. cruzi*, and (iii) *T. brucei* is the most ancient and distantly related of all the trypanosomatids. In this

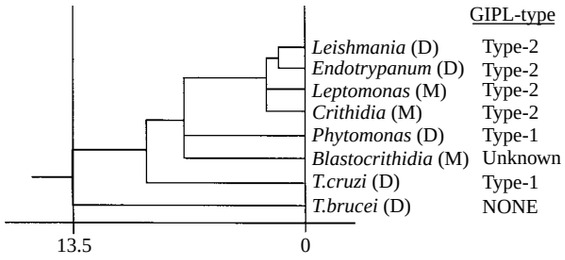

Figure 4. The phylogeny of GIPL types in the trypanosomatids. The phylogenetic tree, based on the average percentage of small-subunit ribosomal RNA divergence (*X* axis), is adapted from Fernandes *et al*. (1993). The monogenetic (M) and digenetic (D) life-cycles are indicated after the species name, followed by the GIPL types expressed by the species. Type-1 indicates exclusively Type-1 GIPLs and Type-2 indicates Type-2 and/or hybrid-GIPLs; see the text for details.

context, it is worth noting that: (i) although *T. brucei* expresses very high levels of protein-linked GPI anchors it does not make free GIPLs, (ii) *T. cruzi* and *Phytomonas* seem to make only Type-1 GIPLs (the type most closely related to protein-linked GPI anchors), and (iii) *Leptomonas*, *Endotrypanum*, *Leishmania* and *Crithidia* make predominantly Type-2 and/or hybrid GIPLs. Thus, one could suggest that the GIPLs have arisen through adaptations of GPI anchor biosynthesis (ubiquitous in the eukaryotes) and that the Type-1 GIPLs preceded the Type-2 GIPLs and related structures such as the *Leishmania* LPGs (figure 4).

There does not seem to be any correlation between the presence or types of GIPL expressed by trypanosomatids and their monogenetic (single host) or digenetic (host and vector) life-cycles. For example, the digenetic parasite *T. brucei* does not express GIPLs, and the monogenetic parasite *Leptomonas* expresses high levels of complex Type-2 GIPLs. The precise functions of cell-surface GIPLs in trypanosomatid parasites are largely unknown. Their abundance suggests that they might have a general protective function by providing a dense, negatively charged, glycocalyx close to the surface of the plasma membrane through which other macromolecules project. However, this function alone does not readily explain the stage- and species-specific nature of GIPL structures.

6. GPI BIOSYNTHESIS

The GPI family of molecules (GPI-anchored glycoproteins and/or the LPGs and/or the GIPLs) are the most abundant molecules on the surface of all the trypanosomatid parasites. Although these molecules show great structural diversity they also share the common core structure, Manα1-4GlcNα1-6*myo*-inositol-1-PO$_4$-lipid. Thus, the biosynthetic enzymes responsible for the synthesis of this common core are attractive targets for the development of anti-trypanosomatid agents. In contrast, some of the unique features of GPI-anchor biosynthesis in the African trypanosomes, such as fatty acid remodelling and inositol acylation/deacylation, offer attractive targets for the

development of trypanocidal agents specific to the VSG-coated African trypanosomes. These issues are discussed in detail in Güther & Ferguson (1995) and Smith *et al*. (1996).

This work was supported by The Wellcome Trust. I thank my many colleagues and collaborators involved in the structural analyses described in this article, especially: Steve Homans, Malcolm McConville, Pascal Schneider, Angela Mehlert, Achim Treumann, Nicole Zitzmann, Andreas Hülsmeier, Chris Redman, Trevor Rutherford, Julia Richardson, Jane Thomas-Oates, Jerry Thomas, Thomas Ilg, Peter Overath, Sam Turco, David Sacks, Sergio Schenkman, Nobuko Yoshida, Luiz Travassos, Alvaro Acosta Serrano, Igor de Almeida, Lucia de Almeida and Norton Heise. The author is a Howard Hughes International Research Scholar.

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