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Lipid metabolism in *Trypanosoma brucei*

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Abstract

Trypanosoma brucei membranes consist of all major eukaryotic glycerophospholipid and sphingolipid classes. These are *de novo* synthesized from precursors obtained either from the host or from catabolised endocytosed lipids. In recent years, substantial progress has been made in the molecular and biochemical characterisation of several of these lipid biosynthetic pathways, using gene knockout or RNA interference strategies or by enzymatic characterization of individual reactions. Together with the completed genome, these studies have highlighted several possible differences between mammalian and trypanosome lipid biosynthesis that could be exploited for the development of drugs against the diseases caused by these parasites.

Keywords

Trypanosoma; Phospholipids; Sphingolipids; Fatty acids; Biosynthesis; Metabolism; Gene IDs

1. Introduction

Parasitic protozoa cause infectious diseases affecting 15% of the global population, with millions of fatalities. One such neglected disease is caused by the protozoan parasite *Trypanosoma brucei*, which causes Human African Trypanosomiasis, also known as African sleeping sickness. According to the World Health Organization, it constitutes a serious health risk to ~60 million people in Sub-Saharan Africa. Due to increased efforts in vector control, the annual rate of infections has fallen to ~50,000 with an estimated ~7000 fatalities per year. In addition, the animal form of the disease, called Nagana, has a devastating economic, social and nutritional impact by affecting the cattle population in Africa. Current human and animal drug therapies are woefully inadequate, expensive, hard to administer, highly toxic and have increasing drug-resistance problems. Hence there is an urgent need for new drugs to treat African sleeping sickness and Nagana, as well as other Third World diseases transmitted by closely related protozoan parasites, such as *Leishmania* spp. and *Trypanosoma cruzi*.

T. brucei belong to the order Kinetoplastida and are considered part of the earliest diverging eukaryotic lineages [1]. As such, they are regarded as a ‘model organism’ for the study of alternative mechanisms by which eukaryotes accomplish basic functions. During their life cycle, trypanosomes encounter the vastly different environments of the mammalian bloodstream and various tissues within the tsetse vector. They respond to these by dramatic

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morphological and metabolic changes, including adaptation of their lipid and energy metabolism [2]. Lipids constitute 11–18% of the dry weight of *T. brucei* and their distribution is consistent with the usual range of lipids found in eukaryotes, such as phospholipids, neutral lipids, fatty acids, isoprenoids, and sterols [3–9]. *T. brucei* bloodstream and procyclic forms contain all major phospholipid classes known in other eukaryotes, accounting for ~80% of membrane lipids [7]. Trypanosomes do not utilise intact phospholipids scavenged from their hosts, but use their repertoire of metabolic and anabolic enzymes to *de novo* synthesise their own phospholipids and glycolipids for their specific requirements [5,10]. Until recently, most of the literature on *T. brucei* lipid metabolism focused on myristate formation and turnover, due to its requirement for glycosylphosphatidylinositol (GPI) anchor biosynthesis and remodeling [11–14].

Completion of the Tri-Tryp (*T. brucei, T. cruzi*, *Leishmania* spp.) genome projects (http://tritrypdb.org/tritrypdb/) has allowed putative identification of homologues of genes necessary for *de novo* biosynthesis of all classes of phospholipids. Recently, several biosynthetic enzymes involved in *de novo* synthesis of phospholipids have been characterized experimentally, including enzymes involved in *de novo* synthesis of fatty acids and the major glycerophospholipid and sphingolipid classes, and several catabolic enzymes, including two phospholipases and a sphingomyelinase (see below). As more enzymes involved in phospholipid metabolism are characterized, our understanding of these parasites will allow direct comparisons with man, and thus new targets are likely to emerge for the development of new anti-protozoan drugs.

2. Lipid composition of African trypanosomes

The total phospholipid composition of *T. brucei* bloodstream and procyclic forms resembles that of other eukaryotic cells. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) represent the most abundant glycerophospholipid classes, comprising 45–60% and 10–20%, respectively, of total phospholipids (Fig. 1). Phosphatidylinositol (PI), phosphatidylserine (PS) and cardiolipin (CL) represent minor glycerophospholipid classes and account for 6–12%, <4% and <3%, respectively, of total lipid phosphorus [6,7,9,15–17]. In addition, *T. brucei* parasites contain substantial amounts (10–15% of total lipid phosphorus) of the sphingophospholipids, sphingomyelin (SM), inositol phosphorylceramide (IPC) and ethanolamine phosphorylceramide (EPC) [6,7,18,19]. Interestingly, trypanosomes from both life cycle stages contain high amounts of ether lipids, which are particularly abundant in PE and PS [7]. A similar observation has also been made in the related kinetoplastids, *Leishmania* spp. ([20–22]; reviewed in [23]), *T. cruzi* [24], and *T. congolense* (P. Bütikofer, E. Greganova, M. Serricchio and A. Acosta-Serrano, unpublished data). Information on the lipid composition of subcellular fractions of *T. brucei* parasites is lacking, except for a recent analysis of the lipid composition of isolated mitochondria from *T. brucei* procyclic forms [25]. A detailed comparative lipidomics analysis of bloodstream and procyclic form *T. brucei* has recently been published [26].

The fatty acyl chain composition of *T. brucei* membrane lipid classes differs between bloodstream and procyclic forms [6,7], possibly reflecting an adaptation of the parasite membrane to the change in environmental conditions in the insect host compared to the mammalian bloodstream [10]. The molecular species composition of PC in bloodstream form parasites was shown to consist of 18:0/18:2 (23%), 16:0/22:6 (12%), 18:0/22:6 (11%), 16:0/18:2 (9%), 18:0/22:4 (7%), 18:2/20:4 and 18:1/20:5 (6%), 18:2/22:5 (5%), 18:0/20:4 (5%), and others (5%) (with the assumption that saturated fatty acids are *sn*-1 esterified) [7,26]. Similar chain lengths and degrees of saturation were also found for the molecular species of PE and PI in bloodstream forms, although the distribution slightly varied. In *T. brucei* procyclic forms, the relative amounts of diacyl glycerophospholipids containing

highly unsaturated fatty acyl chains showed a decrease compared to bloodstream forms, in the order of PC > PE > >PI. *T. brucei* parasites also contain significant proportions of *sn*-1 ether-linked glycerophospholipids, especially in PE (73–84%) and PS (60–88%) [7,15,17,26].

The fatty acyl chain composition of *T. brucei* does not reflect that of their immediate environment. Examination of the fatty acids of *T. b. rhodesiense* bloodstream forms showed that they have a much lower proportion of C16:0 and a higher proportion of long-chain polyunsaturated fatty acids (C18:2, C20:2, C20:4, C22:4, C22:5, C22:6) than in their host plasma, probably as a result of their preferred uptake of these fatty acids from culture medium compared to saturated fatty acids [6]. More recent experiments provided clear evidence for the existence of *de novo* fatty acid synthesis, elongation, and desaturation in *T. brucei* [27,28]. Analysis of the fatty acyl composition of various phospholipids supported the notion that the intrinsic acyl-coenzyme A (acyl-CoA) specificity of the acyltransferases involved in ether- and diacyl-type glycerophospholipid formation, together with the availability of acyl-CoAs, are major factors in producing the final phospholipid molecular species composition. Interestingly, most ether-type species of PC, PE and PI, and most diacyl-type species of PC, PE, PI and PS, contain almost exclusively C18:0 attached to the *sn*-1 position of the glycerol [7,26]. This implies that both the dihydroxyacetonephosphate and glycerol-3-phosphate acyltransferases have either a specificity for C18:0-CoA or, alternatively, are exposed exclusively to C18:0-CoA. In contrast, the *sn*-2 position of the glycerophospholipid subclasses is almost exclusively occupied by unsaturated acyl groups, i.e. predominately C18:2 in the ether-type and mainly C18:2 and C22:4, with some C20:4, in the diacyl-type glycerophospholipids. Presumably, most C20:4 is scavenged from the host plasma and modified to C22:4 by the elongase ELO4 [28].

Like other eukaryotic cells, trypanosome membranes contain substantial amounts of sterols. A recent detailed analysis showed that cholesterol constitutes more that 95% of total sterol in *T. brucei* bloodstream forms isolated from rat blood [29], most of which they take up from the mammalian host by receptor-mediated endocytosis [4,30]. In contrast, *T. brucei* procyclic forms contain a set of sterols, with cholesta-5,7,24-trienol, cholesterol and ergosta-5,7,25(27)-trienol representing the most abundant species, accounting for >84% of total sterol [29]. Some of the sterols in *T. brucei* may accumulate in lipid rafts [31], which seem enriched in the flagellar membrane [32].

3. Uptake and synthesis of lipid precursors

3.1. Uptake of components for de novo lipid biosynthesis

It has long been known that trypanosomatids scavenge lipids from their host environment. This may occur via uptake of protein-bound fatty acids and *lyso*-phospholipids, which are then assembled to phospholipids, or by receptor-mediated endocytosis of lipoprotein particles [5,33–36]. In addition, it has been shown that *T. brucei* bloodstream forms in culture require the uptake of lipids from the medium for optimal growth [34,37]. However, more recent studies have demonstrated that *T. brucei* bloodstream and procyclic forms are capable of *de novo* synthesis of all major phospholipid classes using exogenous lipid precursors [15–19,38]. In addition, *T. brucei* parasites possess a system for *de novo* synthesis of fatty acids [28].

Choline, ethanolamine and *myo*-inositol represent components of the head groups of the major glycerophospholipid and sphingolipid classes. Although choline uptake activities have been reported in certain protozoan parasites [39,40], *T. brucei* bloodstream forms show no transport activity for choline and, instead, meet their demand for choline by uptake of *lyso*-PC from the culture medium, or the host plasma [41,42]. In contrast, efficient uptake of

ethanolamine, followed by incorporation into the cellular PE pool, has been reported in both bloodstream and procyclic form *T. brucei* [15–17,42]. A transporter for ethanolamine has, however, not been identified in protozoa. In contrast, several *myo*-inositol transporters have been reported and characterized in *L. donovani* [43–46]. In addition, *myo*-inositol uptake has also been documented in *Trypanosoma cruzi* [47,48] and *T. brucei* bloodstream forms [38].

Serine, an essential precursor for sphingolipid and PS synthesis, is readily taken up by *T. brucei* bloodstream and procyclic forms and incorporated into the respective lipid classes [15,17,18].

3.2. De novo fatty acid synthesis

A type II prokaryotic-like fatty acid synthesis system located inside the mitochondria was initially thought to be the source of *de novo* synthesised fatty acids in *T. brucei* [27]. However, the low level of fatty acid production by this system suggested that, in addition to fatty acid scavenging from the host, there must be another *de novo* synthetic route to myristate (C14:0). Subsequently, it was shown that four microsomal elongases with different specificities produced nearly all of the *de novo* synthesised fatty acids in *T. brucei* (Table 1) [28,49]. The first two elongases convert C4:0 to C10:0 and C10: to C14:0, respectively, while elongase 3 extends C14:0 to C18:0 (Fig. 2). This last enzyme is stage specifically down-regulated in bloodstream form trypanosomes, resulting in the almost exclusive production of myristate (C14:0), which is utilized for GPI anchor synthesis and remodeling [13]. Finally, elongase 4 likely elongates host-derived long chain polyunsaturated fatty acids, i.e. C20:4 to C22:4 [49], which may then be desaturated further by a plant-like fatty acid desaturase to form significant amounts of C22:5 and C22:6 [50] (Table 1).

3.3. Fatty acid activation

Fatty acids normally undergo activation to CoA derivatives prior to their utilization in anabolic and catabolic pathways, such as core glycerophospholipid biosynthesis, phospholipid reacylation, fatty acid elongation, cholesterol ester formation, and β-oxidation. Activated fatty acids also serve a variety of protein targeting and regulatory roles including protein acylation, enzyme activation/inhibition, cell signaling, and protein transport [51,52]. *N*-myristoylation in *T. brucei* has been validated as drug target [53] and efforts by academia are now being made to obtain drug-like lead compounds [54].

Activation of fatty acids is generally accomplished using a family of enzymes, termed fatty acyl-CoA synthetases (ACSs). Five out of eight potential *T. brucei* ACSs have been characterised (Fig. 2, Table 1). TbACS1–4 are found in a tandem array and are constitutively expressed in both main life cycle stages, with TbACS3 being the dominant form. TbACS1, TbACS3, and TbACS4 show specificity towards C12:0–C14:0, C14:0–C16:0, and C14:0–C18:0, respectively, while TbACS2 prefers C10:0 [55]. TbACS5 is not essential in either of the main life cycle stages, despite it having a preference for C14:0 (G.S. Richmond and T.K. Smith, unpublished data).

3.4. Dihydroxyacetonephosphate and glycerol-3-phosphate formation

A significant proportion of the glycerophospholipids have ether-linked aliphatic, i.e. alkyl or alk-1-enyl, chains attached to the *sn*-1 position of the glycerol backbone (see above). However, their function(s) and essentiality in *T. brucei* are unknown. Ether-linked lipids are *de novo* synthesised from dihydroxyacetonephosphate, via acyl-CoA:dihydroxyacetonephosphate acyl-transferase, alkyl-dihydroxyacetonephosphate synthase and alkyl-dihydroxyacetonephosphate oxidoreductase [56] (Fig. 3, Table 1). The putative enzymes in *T. brucei* have been shown to be associated with glycosomal fractions

[57,58]. To date, only the alkyl-dihydroxyacetonephosphate synthase has been recombinantly expressed and characterized [59].

3.5. De novo ceramide synthesis

Ceramide, the hydrophobic precursor of sphingolipids, consists of a sphingoid long-chain base to which a fatty acyl chain is attached via an amide bond. Its synthesis occurs in the endoplasmic reticulum (ER) and starts with the conversion of serine and fatty acyl-CoA into 3-ketosphinganine by serine palmitoyltransferase complex (reviewed by [60,61]). Subsequently, the reaction product is reduced to sphinganine by 3-ketosphinganine reductase. Sphinganine can now enter two pathways, the phosphorylation to the lipid mediator, sphinganine-1-phosphate, or the acylation to dihydroceramide, which in turn, becomes desaturated to ceramide (Fig. 2, Table 1).

Experimental evidence that the pathway of *de novo* ceramide synthesis is active in *T. brucei* has been obtained by labeling procyclic trypanosomes in culture with radioactive serine, which is readily incorporated into sphingolipids [15,18]. Recently, a similar observation has also been made in *T. congolense* procyclic forms in culture (P. Bütikofer, E. Greganova and A. Acosta-Serrano, unpublished data). A search of the *T. brucei* genome reveals candidate genes for ceramide biosynthesis (Table 1). Both *T. brucei* bloodstream and procyclic forms clearly depend upon *de novo* sphingoid base synthesis, as was demonstrated by blocking serine palmitoyltransferase in these life cycle stages, resulting in growth inhibition and delayed kinetoplast segregation and aberrant cytokinesis, respectively [18,62]. In contrast, *Leishmania* parasites lacking serine palmitoyltransferase are viable as long as they are substituted with ethanolamine, a metabolic product of sphingoid base degradation [63,64].

4. The Kennedy pathway

4.1. CDP-ethanolamine branch

PE, the second most abundant glycerophospholipid class in *T. brucei* bloodstream and procyclic forms, can be generated via its CDP-activated intermediate, CDP-ethanolamine, by a reaction sequence termed the ‘Kennedy pathway’, after its original discovery by Kennedy and Weiss [65]. It involves the phosphorylation of ethanolamine by ethanolamine kinase, followed by activation of ethanolamine-phosphate to CDP-ethanolamine via ethanolamine-phosphate cytidylyltransferase. Finally, the activated head group is transferred to diradylglycerol by ethanolamine phosphotransferase (Fig. 3, Table 1). The first two reactions are catalyzed by cytosolic enzymes, whereas the third step is mediated by an integral membrane protein of the ER (reviewed by [66]).

All enzymes involved in PE formation by the ‘Kennedy pathway’ in *T. brucei* have been identified and experimentally confirmed [15–17,67]. Disruption of the CDP-ethanolamine branch in procyclic forms using RNA interference (RNAi) resulted in severe growth phenotypes and revealed dramatic changes in cellular PE, PC and PS levels [15,16]. In addition, a block in PE synthesis caused alterations in mitochondrial morphology and the formation of multinucleate parasites [68]. Similar observations were also made in *T. brucei* bloodstream forms after knocking out ethanolamine-phosphate cytidylyltransferase, which validates the Kennedy pathway as a potential drug target [17].

4.2. CDP-choline branch

A similar pathway, involving CDP-activated choline, also leads to the formation of PC, the most abundant phospholipid class in eukaryotic cells, including *T. brucei*. Candidate trypanosome genes for all three enzymes of the CDP-choline branch of the ‘Kennedy pathway’ have been identified (Fig. 3, Table 1). The first and third enzymes, choline kinase

[67] and choline phosphotransferase [15], have been characterized experimentally and were found to show dual specificities for choline and ethanolamine, and CDP-choline and CDP-ethanolamine, respectively. Choline kinase is essential in *T. brucei* bloodstream forms (S.A. Young, F. Gibellini and T.K. Smith, unpublished data), indicating the importance of this pathway in choline metabolism of the parasite. The second enzyme, choline-phosphate cytidylyltransferase, which, in analogy to its homologues in mammalian cells or to *T. brucei* ethanolamine-phosphate cytidylyltransferase in the CDP-ethanolamine branch, likely represents the rate-limiting reaction (S.A. Young and T.K. Smith, unpublished data).

4.3. Cross-talk between the two branches

An alternative route for PC synthesis in eukaryotic cells involves methylation of PE to PC, catalyzed by phosphatidylethanolamine *N*-methyltransferases [66]. However, in contrast to *Leishmania*, the genomes of *T. brucei* and *T. cruzi* show no homologues for these enzymes. In addition, labeling of *T. brucei* bloodstream and procyclic forms with radiolabeled and stable isotope labeled ethanolamine, or serine, showed no evidence for the conversion of PE to PC [15,17,26,42] (T.K. Smith and P. Bütikofer, unpublished data), indicating that this pathway is absent in trypanosomes. This evidence suggests that *T. brucei* are auxotrophic for choline, i.e. they depend on choline scavenged from the host. Interestingly, experiments involving RNAi-mediated gene silencing of ethanolamine phosphotransferase revealed that a block in PE synthesis via the CDP-ethanolamine branch led to increased PE formation via choline phosphotransferase, which shows dual specificity for the substrates of both branches (see above), thereby changing the molecular species composition of PE from mostly alk-1-enyl-acyl-type species to mostly diacyl-type species [15]. Thus, parasites try to compensate for a lack of one subclass of PE by up-regulating another subclass, indicating that the PE content of *T. brucei* is tightly regulated, and essential, for its survival.

5. PS formation and metabolism

5.1. PS synthesis

In prokaryotes and yeast, PS is formed from serine and CDP-diacylglycerol in a reaction catalyzed by PS synthase [69]. In contrast, in mammalian cells, PS synthesis is mediated by two serine-exchange enzymes, PS synthase-1 (PSS1) and PS synthase-2 (PSS2). In plants, both pathways have been identified (reviewed by [70]). PSS1 and PSS2, which are localized in mitochondria-associated membranes, differ in their substrate specificity. Whereas PSS1 utilizes PC for the head group exchange reaction, PSS2 is specific for PE as substrate. The two enzymes share 32% amino acid identity and are membrane bound via multiple transmembrane domains [70]. Studies using knockout mice showed that deletion of either *PSS1* or *PSS2*, but not both, is compatible with mouse viability [71–73].

In *T. brucei*, PS represents a minor membrane component [7,15]. Its biosynthetic pathway has not been firmly established [15,17]. A candidate gene encoding a putative PS synthase, or a PSS2, has been identified in the *T. brucei* genome (Fig. 3, Table 1). Experiments to establish the route for PS formation in *T. brucei* are currently under way in our laboratories.

5.2. PS metabolism

In both prokaryotes and eukaryotes, PS is used as the only (in most bacteria) or primary (in most other cell types) source for PE formation via PS decarboxylation ([74]; recently reviewed by [75]). The reaction is catalyzed by two types of enzymes, PS decarboxylase I, comprising bacterial and mitochondrially localized enzymes, and PS decarboxylase II, comprising enzymes associated with the endomembrane system. The two types of enzymes share a highly conserved GST motif in the C-terminal region of the proteins, which is the autocatalytic cleavage and processing site for the formation of the α- and β-subunits [76,77].

Although PS decarboxylases are present in many prokaryotic and eukaryotic organisms, the relative contribution of PS decarboxylation to cell viability, or function, greatly varies [75].

In *T. brucei*, decarboxylation of PS to PE has been shown to occur in procyclic forms [15]. However, the pathway contributes little to PE formation since depletion of cellular PE in procyclic trypanosomes by disrupting the CDP-ethanolamine branch of the Kennedy pathway could not be compensated by increased PE formation via decarboxylation of PS [15]. In addition, stable isotope labelling experiments showed virtually no PE formation from PS in *T. brucei* bloodstream forms in culture [17]. A gene encoding a putative PS decarboxylase I has been identified in the *T. brucei* genome (Fig. 3, Table 1) and was found to be expressed in both main life cycle stages. In addition, recombinant *T. brucei* PS decarboxylase I has been shown to be correctly processed and catalytically active, implying its activity may be highly regulated or restricted in the parasite (T.K. Smith, unpublished data).

6. Sphingolipid metabolism

6.1. Biosynthesis of sphingophospholipids

The hydrophilic head groups for the formation of SM, IPC and EPC, are transferred to their common hydrophobic precursor lipid, ceramide, from the glycerophospholipids PC, PI and PE, respectively. Two SM synthases have been identified in the Golgi and the plasma membrane of mammalian cells [78–80] and an IPC synthase has been found in the Golgi of the yeast, *S. cerevisiae* [81]. In addition, activities responsible for EPC formation have been described in mammalian cells [82–84].

In *T. brucei*, a sphingolipid synthase (SLS) gene family consisting of four tandemly linked genes has recently been identified (Fig. 2, Table 1) [19]. The deduced enzymes show very high degrees of amino acid identity between each other and are predicted to contain multiple transmembrane domains [19,85]. RNAi against a nucleotide sequence common to all four putative SLS genes showed that sphingolipid synthesis is essential for growth of *T. brucei* bloodstream forms in culture [19,86]. However, the SLS-depleted parasites showed little decrease in cellular SM and EPC levels, suggesting that the turnover of these lipids may be very low. Expression of the *T. brucei* SLS4 gene in *Leishmania major* promastigotes demonstrated that its product has SM and EPC synthase activity [19]. This finding is in contrast to a report claiming that the product of the SLS4 gene is involved in SM and IPC formation [86]. Interestingly, detailed sphingolipid analysis revealed that *T. brucei* procyclic forms contain significant amounts of SM and IPC, while bloodstream forms lack IPC but contain small amounts of EPC [19], suggesting that the four putative SLSs may be differentially expressed in the main life cycle forms and likely encode enzymes with different substrate specificities. This has recently been confirmed by showing that stumpy bloodstream forms (i.e. parasites differentiating from bloodstream to procyclic cells) contain IPC and that the expression of the corresponding SLS is increased in this life cycle form [87]. Recent unpublished data using a cell-free expression system revealed that SLS1 and 2 primarily synthesise IPC and EPC, respectively, while SLS3 and 4 are bifunctional SM/EPC synthases (J.D. Bangs, personal communication). A similar dual substrate specificity for SM and EPC formation has recently been described for SLS2 in mammalian cells [88].

6.2. Catabolism

Intracellular degradation of sphingolipids is achieved by sphingomyelinases (SMases), which catalyse sphingolipid hydrolysis to ceramide and a corresponding head group, i.e. phosphorylcholine in the case of SM degradation. Various SMases have been described, differing in subcellular localisation and tissue specificity. In many cases, SMases are

activated by growth factors, cytokines, chemotherapeutic agents, irradiation, nutrient removal and other related stress factors (reviewed by [89,90]).

The *T. brucei* genome contains candidate genes for two putative acidic SMases and one neutral SMase (Fig. 2, Table 1). The neutral SMase, which is homologous to the yeast enzyme, Isc1, has recently been found to play a crucial role in the exocytic flux of variant surface glycoprotein (VSG) to the cell surface, i.e. flagellar pocket (S.A. Young and T.K. Smith, unpublished data). In *Leishmania*, SMase was shown to be involved in the degradation of host SM [91].

As mentioned above, in *Leishmania* promastigotes ceramide degradation by sphingosine-1-phosphate lyase (Fig. 2, Table 1) has been shown to be essential for growth. Apparently, this pathway is required for the formation of ethanolamine-phosphate, a direct product of the reaction, for anabolic processes [64]. Preliminary data indicate that this enzyme is also functional in *T. brucei* procyclic forms (J. Jelk and P. Bütikofer, unpublished data).

7. CDP-DAG synthesis

CTP and phosphatidic acid are utilized by the rate limiting and tightly regulated enzyme, CDP-diacylglycerol (CDP-DAG) synthase, to form CDP-DAG, which is a central metabolite in the *de novo* synthesis of PI, PS, PG and CL. Genes encoding CDP-DAG synthases have been cloned from several organisms and show high sequence identity, suggesting conservation during evolution. Homologues of CDP-DAG synthases have been found in all eukaryotic organisms sequenced to date and some appear to have multiple copies. Mammals, for example, appear to have two copies, which are differently expressed and regulated [92–95].

In *T. brucei*, a putative CDP-DAG synthase gene has recently been identified (Table 1), and its product was found to be essential in both bloodstream and procyclic forms of the parasite (A. Lilley and T.K. Smith, unpublished data). Interestingly, the related organisms *T. cruzi* and *Leishmania* appear to have two CDP-DAG synthase genes, one clustering with the *T. brucei* homologue in phylogenetic analysis and one clustering with bacterial homologues.

The previous observation that the *T. brucei* PI synthase is located in the ER and Golgi [38,96] (see below) suggests that CDP-DAG may be also be synthesised in both organelles, or transported from one to the other. In addition, since CDP-DAG is also a precursor for PG and CL synthesis, which most likely takes place in mitochondria, where these lipids are required to maintain organelle integrity, membrane potential and function (see below), CDP-DAG must either be synthesized in, or transported to, the mitochondria.

8. PG and CL synthesis

CL, also called diphosphatidylglycerol, represents a component of the plasma membrane of many Gram-positive and Gram-negative bacteria and the inner mitochondrial and chloroplast membranes of eukaryotes. Due to its chemical structure, consisting of four acyl chains and a small negatively charged head group, CL has the tendency to form non-bilayer structures and can organize into membrane sub-domains (recently reviewed by [97,98]). In addition, CL plays an important role in the stabilization and function of many mitochondrial proteins or protein complexes (recently reviewed by [99,100]).

The synthesis of CL in both prokaryotes and eukaryotes starts with the activation of phosphatidic acid by CTP (recently reviewed by [101]). Subsequently, the phosphatidyl group is transferred to the *sn*-1 hydroxyl group of glycerol-3-phosphate to form phosphatidylglycerophosphate (PGP), which becomes dephosphorylated to

phosphatidylglycerol (PG). In prokaryotes, PG receives the additional phosphatidyl group from another PG molecule, whereas in eukaryotes, the phosphatidyl group is transferred from phosphatidyl-CMP. The prokaryotic CL synthase catalyzes the reaction by a phospholipase D-type transesterification mechanism that is fully reversible (reviewed by [102]) and, thus, may also be involved in CL degradation. In contrast, the eukaryotic enzyme catalyzes CL formation by an irreversible phosphatidyl-transferase mechanism, a type of reaction that is also involved in PS, PI and PG synthesis. In *Escherichia coli*, CL synthase is bound to the inner membrane, with the catalytic site facing the periplasm [103], whereas in rat liver mitochondria, it localizes to the inner membrane and the active site faces the matrix [104].

In *T. brucei* procyclic forms, CL has been identified in isolated mitochondria and characterized using mass spectrometry, revealing several molecular species [25]. In contrast, at present no information is available on the pathway for PG and CL formation. However, the genome of *T. brucei* shows candidate genes encoding putative PGP synthase and CL synthase (Fig. 3, Table 1). Preliminary data indicate that both enzymes are essential for normal growth in *T. brucei* procyclic forms (M. Serricchio and P. Bütikofer, unpublished data).

9. Inositol phospholipid metabolism

9.1. PI synthesis

PI is a ubiquitous eukaryotic phospholipid, which serves as a precursor for the phosphorylated PI species (PIPs), second messenger molecules and GPI anchors. PI is synthesised via the action of a PI synthase using *myo*-inositol and CDP-DAG and releasing CMP, and has been studied in such organisms as *S. cerevisiae*, *Arabidopsis thaliana* and *Toxoplasma gondii* [105–107].

The conundrum that bloodstream form GPI anchors cannot be in vivo labelled with [^{3}H]inositol led to the discovery that *de novo* synthesis was responsible for supplying *myo*-inositol for PI, and GPI, synthesis in the ER (Figs. 3 and 4, Table 1) [96]. This observation was confirmed by immunofluorescence studies showing that the PI synthase is localized in the ER and the Golgi, where it utilizes imported extracellular inositol to generate the majority of bulk PI in the parasite [38].

9.2. Formation of PIPs

In many eukaryotes, trafficking, endocytosis, and Golgi maintenance are PI-mediated events (reviewed by [108]). PIPs are known to effect proteins of eukaryotic components of secretory pathways, some of which have been identified in *T. brucei*, i.e., clathrin, adapter proteins, and Rab GTPases [109].

Several putative PI kinases have been identified in the genome (Fig. 3, Table 1), however the apparent lack of genes encoding class I or II PI 3-kinases suggests that *T. brucei* may not have PI 3-kinase-dependent signaling pathways. However, an identified class III PI 3-kinase has been implicated in Golgi segregation and endocytotic trafficking [110]. The *T. brucei* database also contains genes for two putative PI 4-kinases, TbPI4KIII-α and TbPI4KIII-β, the latter of which is required for maintenance of Golgi structure, protein trafficking, normal cellular shape, and cytokinesis in procyclic form trypanosomes [111]. In addition, the *T. brucei* genome reveals four putative PI monophosphate kinases, a type I PI4P 5-kinase, a type III PI3P 5-kinase (PIKFYVE), and two type II PI monophosphate kinase isoforms. Collectively, the presence of these putative enzymes suggests that *T. brucei* can, in principle, synthesize all of the mono- and bisphosphorylated PI species. Recently, these classes of PIPs have been identified in a comprehensive lipidomics analysis (T.K. Smith, unpublished data).

10. GPI biosynthesis

T. brucei bloodstream forms avoid the hosts' innate immune system by undergoing antigenic variation, which involves switching of GPI-anchored VSGs [112]. Despite the variation in the VSG protein, the GPI core structure attached to protein remains unchanged and comprises of ethanolamine-PO_4-6Manα1-2Manα1-6Manα1-4GlcNα1-6D-*myo*-inositol-1-PO_4-dimyristoylglycerol [113]. The biosynthesis of GPI anchors has been validated both genetically and chemically as a potential therapeutic drug target in bloodstream form *T. brucei* [114–117].

GPI biosynthesis and attachment to protein has been elucidated in bloodstream form *T. brucei*, mainly through the use of a cell-free system consisting of washed membranes in the presence of either UDP-GlcNAc or synthetic substrate analogues and radiolabelled GDP-mannose, allowing the stepwise formation of radiolabelled GPI intermediates (Fig. 4, Table 1) ([118–122]; reviewed in [123,124]). The initial step is catalysed by a sulfhydryl dependent multi-protein complex, which transfers GlcNAc from UDP-GlcNAc to PI to form GlcNAc-PI. The PI utilized for GPI biosynthesis in the ER is made almost exclusively from *de novo* synthesized inositol [96]. GlcNAc-PI is then de-N-acetylated to form GlcN-PI [125–127], and is a pre-requisite for further processing, i.e. mannosylation. Numerous substrate analogues of GlcNAc-PI have allowed the specificity of this zinc dependent metalloenzyme to be explored, leading to the formation of parasite specific potent suicide inhibitors [117,128,129]. Subsequent mannosylation of GlcN-PI involves three distinct dolichol-phosphate-mannose dependent mannosyltransferases. In *T. brucei*, unlike most other eukaryotic GPI pathways, the addition of the first mannose to GlcN-PI precedes inositol acylation (reviewed in [123,124]). Inositol acylation is an essential pre-requisite for the transfer of ethanolamine-phosphate from PE to the 6-hydroxyl group of the third mannose of Man_3-GlcN-(acyl)PI. Again various synthetic substrate analogues have been used to explore the specificity of the mannosyltransferases and the inositol acyltransferase (reviewed by [130]). Unusually, inositol acylation and subsequent inositol deacylation are inhibited specifically by the serine protease inhibitors, phenylmethylsulfonyl fluoride and diisopropylfluorophosphate, respectively [122,131]. The GPI precursor, EtN-P-Man_3-GlcN-PI, is then subjected to fatty acid remodeling [11], where a stepwise replacement of the acyl chains with myristate is mediated by two phospholipases and two myristoyl-CoA dependent acyltransferases, resulting in the mature GPI, EtN-P-Man_3-GlcN-(dimyristoyl-)PI. Subsequently, this mature GPI is covalently attached to VSG, mediated by the *T. brucei* transamidase complex [132]. A specific C-terminal GPI addition signal peptide is cleaved, forming a new C-terminus, by activation of the carbonyl group of the ω amino acid, allowing nucleophilic attack on the activated carbonyl by the amino group of the ethanolamine-phosphate linked to the third mannose of the mature GPI precursor forming a new amide linkage [133]. Finally, mature GPI-anchored VSG destined for exocytosis to the flagellar pocket, via the recycling endosomes, undergoes a process called myristate exchange, whereby the existing myristates of the GPI anchor are replaced with two new myristates [12,14]. A candidate gene product, TbGup1, involved in the addition of myristate to the sn-2 position of the glycerol backbone during lipid remodeling of GPI lipids and proteins, has been reported [134].

11. Other pathways involved in lipid homeostasis

11.1. N-glycosylation pathway

All variants of VSG protein are GPI-anchored and modified with one to three N-glycans. Seminal work by the Ferguson group [135,136] has shown that *T. brucei* parasites are unusual amongst eukaryotes, as they are able to transfer *en bloc* oligomannose structure or biantennary structures by the different homologues of the oligosaccharyltransferase STT3

subunits identified in the genome in a site-specific manner, i.e. $Man_9GlcNAc_2$-PP-Dol for Asn-428 and $Man_5GlcNAc_2$-PP-Dol for Asn-263 of VSG221 (Fig. 5, Table 1) [137]. Interestingly, some of the glycosyltransferases involved in making and processing high mannose structures are not essential [135,136]. However, activation of mannose as either GDP-mannose or dolichol-phosphate-mannose is essential in the bloodstream form of the parasite [138,139] (J. Nunes and T.K. Smith, unpublished data). Interestingly, unlike in most other eukaryotes, the recycling of Dol-PP to Dol-P by the gene product of CHW8 (Table 1) is essential in *T. brucei* (T. Chang and T.K. Smith, unpublished data).

11.2. Mevalonate pathway

The mevalonate pathway is a target for drug intervention to treat many diseases, since it provides the essential five carbon building blocks used in the biosynthesis of several ubiquitous families of essential molecules, such as sterols, dolichols, ubiquinones, carotenoids and prenylated proteins, that are essential for many cellular functions (reviewed by [140]). Humans and trypanosomes share the same pathway for isoprenoid biosynthesis, however, the *T. brucei* genome reveals that the trypanosome mevalonate pathway is more related to that found in bacteria and archae than that in eukaryotes (Fig. 6, Table 1).

All isoprenoid compounds are synthesised from isopentenyl-diphosphate and dimethylallyl diphosphate, the later being formed from the former by isopentenyl pyrophosphate isomerase. Preliminary findings suggest that the *T. brucei* isopentenyl pyrophosphate isomerase is a Type II, riboflavin-dependent isomerase closely related to archae homologues (T. Chang and T.K. Smith, unpublished data). In addition, both the isopentenyl pyrophosphate isomerase and the mevalonate-diphosphate decarboxylase have been found to be essential in *T. brucei* bloodstream forms, thus for the first time validating the *T. brucei* mevalonate pathway as a drug target (T. Chang, E. Byres, W.N. Hunter and T.K. Smith, unpublished data). Crystal structures for two of the trypanosomatid enzymes have recently been reported, possibly allowing future *in silico* drug design [141,142]. Localisation of the components of the pathway seems to be different between trypanosomatids, but *T. brucei* hydroxymethylglutaryl-CoA reductase activity is associated with the mitochondria [143]. Several drugs targeting enzymes within the mevalonate pathway have been shown to have anti-parasitic activity [35,144,145]. Trypanosomes have an active isoprenoid metabolism, which differs in several aspects from mammals, in that they make dolichols of 11 and 12 isoprene units, ubiquinone with 9 isoprene units and a series of sterols. Bloodstream form trypanosomes acquire most of the cholesterol from their mammalian host via receptor-mediated endocytosis of low density lipoproteins [4,30]. Procyclic form trypanosomes, instead, are known to *de novo* synthesize a set of unconventional sterols [29], and several enzymes involved in sterol synthesis in trypanosomatids have recently been identified [146–149]. Since 25-azalanosterol, an inhibitor of sterol methyltransferase, was found to inhibit growth of both bloodstream and procyclic form *T. brucei* parasites, sterol biosynthesis seems essential in both life cycle stages [29]. In addition, bisphosphonate inhibitors of farnesyl diphosphate synthase, which was shown to be essential in *T. brucei* [150], inhibit proliferation of *T. brucei* [151]. Furthermore, extensive studies on protein prenylation, mediated by farnesyltransferase and two geranylgeranyltransferases, have shown that protein prenylation is essential in *T. brucei* (reviewed in [152,153]). The utilization of ‘piggy-back medicinal chemistry’ of anti-cancer research in this area may lead to the identification of promising lead compounds [152,154].

11.3. Prostaglandin formation

In higher eukaryotes, bioactive precursors stored in glycerophospholipids and released by phospholipases have been recognized as important factors in signal transduction and in the generation of lipid mediators [155,156]. Phospholipase-modified membrane lipids are

themselves important mediators in cellular processes, such as apoptosis, membrane trafficking and transport [157,158].

The fate of unsaturated fatty acids liberated from glycerophospholipids in *T. brucei* has not been studied in much detail. However, free arachidonic acid has been implicated in regulating calcium mobilization in procyclic form trypanosomes [159,160] and, in addition, has been shown to serve as a precursor for prostaglandin biosynthesis in *T. brucei* [161]. Although a putative mechanism for the release of arachidonic acid from *T. brucei* glycerophospholipids has been proposed [162], the phospholipases mediating these events in *T. brucei* are not known.

11.4. Phospholipases

Phospholipases form a diverse series of enzymes that exist in almost every type of cell and are optimized to hydrolyze glycerophospholipids at specific ester bonds. Despite their considerable variation in structure and function, they can be classified into two sets, i.e. the acyl hydrolases and the phosphodiesterases, according to the cleavage of the ester bond for which they are specific. Phospholipase A_1, phospholipase A_2, phospholipase B, and lysophospholipase $A_{1/2}$ constitute the acyl hydrolases, whereas the phosphodiesterases are represented by phospholipase C and phospholipase D. Three general functions can be ascribed to the physiologic relevance of phospholipases: they can (1) serve as digestive enzymes, i.e. A-type phospholipases are ubiquitous in snake and vespid venoms, (2) play important roles in membrane maintenance and remodeling, i.e. fatty acyl chains of glycerophospholipids can be cleaved and exchanged by acyl hydrolases and acyltransferases, respectively, and (3) regulate important cellular mechanisms, i.e. generate bioactive lipid molecules used in signal transduction.

To date, only two phospholipases have been characterized in *T. brucei* (Table 1), the GPI-hydrolyzing phospholipase C (GPI-PLC) and a PC-specific phospholipase A_1 (PLA_1). GPI-PLC is a C-type phospholipase implicated in the cleavage of the VSG GPI anchor, generating DAG and a 1,2-cyclic inositol-phosphate containing epitope linked to the GPI head group of VSG [163–165]. For many years, the subcellular localization of GPI-PLC in *T. brucei* bloodstream forms presented a topological problem for its putative role in VSG release from the surface of bloodstream form parasites during differentiation to procyclic forms. Work by several laboratories indicated that GPI-PLC faces the cytosol, or is localized in intracellular vesicles [166–169]. However, in a recent report, GPI-PLC has been localized to (a restricted area of) the plasma membrane [170], which is consistent with an earlier report [171], resolving the topological issue since the enzyme and its major substrate are located in the same compartment. Nevertheless, the biological role for GPI-PLC as a VSG lipase remains unclear, as the enzyme cannot be active in bloodstream form trypanosomes under normal conditions because it would hydrolyze and release the protective VSG surface coat.

The only other identified and characterised phospholipase from *T. brucei* is a cytosolic PLA_1, which acts on PC and releases long polyunsaturated fatty acids (Fig. 2, Table 1) [172,173]. The gene of this enzyme may be a result of horizontal gene transfer from the proteobacterium *Sodalis glossinidius*, an intracellular endosymbiont of *Glossina* (tsetse) flies, the insect vector host of *T. brucei*.

The apparent absence of a *T. brucei* phospholipase D homologue, which normally catabolises PC and/or SM in other organisms, has led to the hypothesis that the action of a phospholipase D may have been substituted by the concerted actions of a PLA_1 and a lysophospholipase A, the later of which has been characterized (T.K. Smith, unpublished data). Unfortunately, neither of these lipases are essential in *T. brucei* bloodstream and procyclic

forms, implying the parasites may have an alternative means to catabolise PC, perhaps via the concerted actions of SM synthases and SMase.

Perspectives

In recent years, since the completion of the Tri-Tryp genomes, considerable progress has been made in the molecular biological characterisation of several lipid biosynthetic pathways. However many more genes and their products require detailed genetic and biochemical validation, including detailed enzymological studies, before we can take advantage and exploit possible differences in these pathways between humans and eukaryotic pathogens, such as *T. brucei*, towards the development of drugs against the diseases they cause.

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Abbreviations

ACS	fatty acyl-CoA synthetase
CoA	coenzyme A
CL	cardiolipin
DAG	diacylglycerol
ER	endoplasmic reticulum
EPC	ethanolamine phosphorylceramide
GPI	glycosylphosphatidylinositol
GPI-PLC	glycosylphosphatidylinositol phospholipase C
IPC	inositol phosphorylceramide
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PG	phosphatidylglycerol
PGP	phosphatidylglycerophosphate
PI	phosphatidylinositol
PIPs	phosphorylated PI species
PS	phosphatidylserine
PSS1	PS synthase-1
PSS2	PS synthase-2
PLA1	phospholipase A1
SM	sphingomyelin
SMase	sphingomyelinase

SLS sphingolipid synthase

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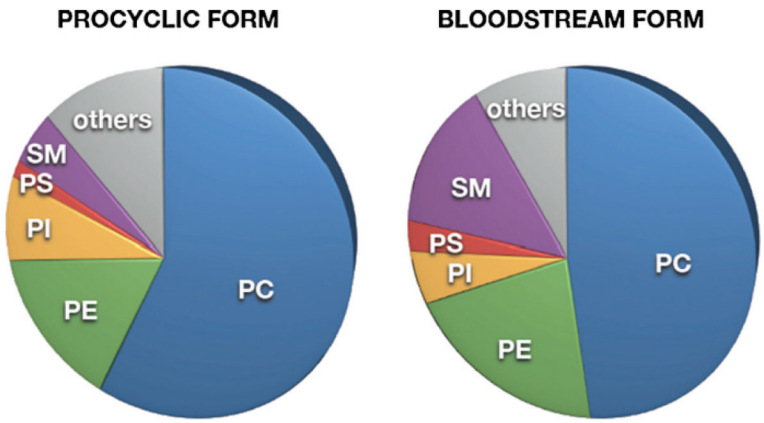

Fig. 1.

Phospholipid composition in *T. brucei*. Relative distribution of the phospholipid classes, PC, PE, PI, PS, and SM, in *T. brucei* procyclic and bloodstream forms. Others include IPC, EPC, CL, PG, PIPs, phosphatidic acid, *lyso*-phospholipids, phosphorylated prenyls, Dol-Ps.

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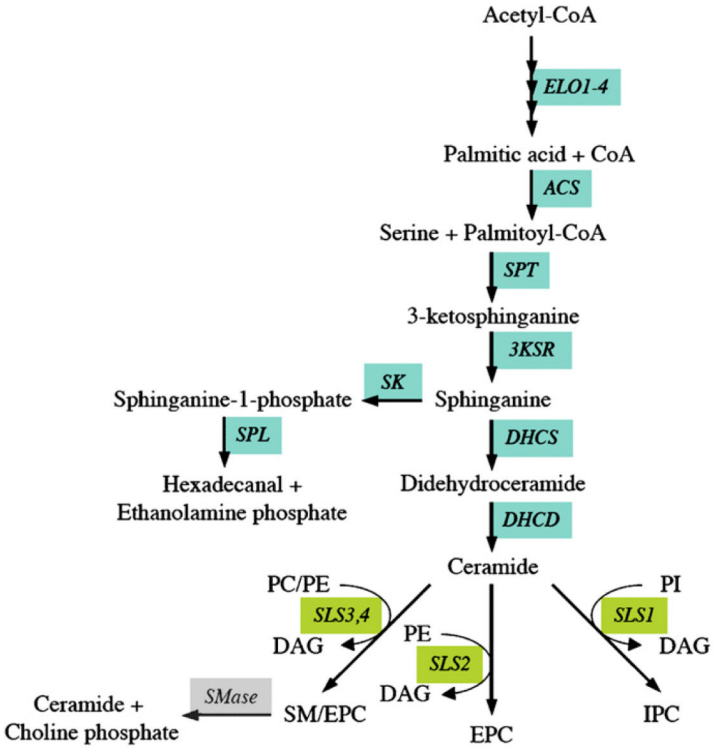

Fig. 2.

Predicted pathways for sphingolipid biosynthesis in *T. brucei*. Enzymes for which candidate genes have been identified in the *T. brucei* gene DB are indicated; see Table 1 for the abbreviations of the enzymes.

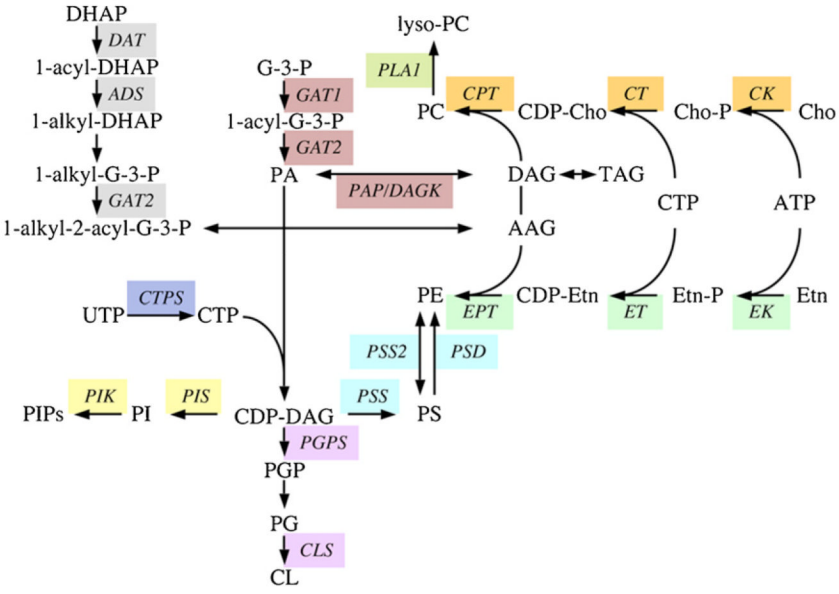

Fig. 3.

Predicted pathways for glycerophospholipid biosynthesis in *T. brucei*. Enzymes for which candidate genes have been identified in the *T. brucei* gene DB are indicated; see Table 1 for the abbreviations of the enzymes.

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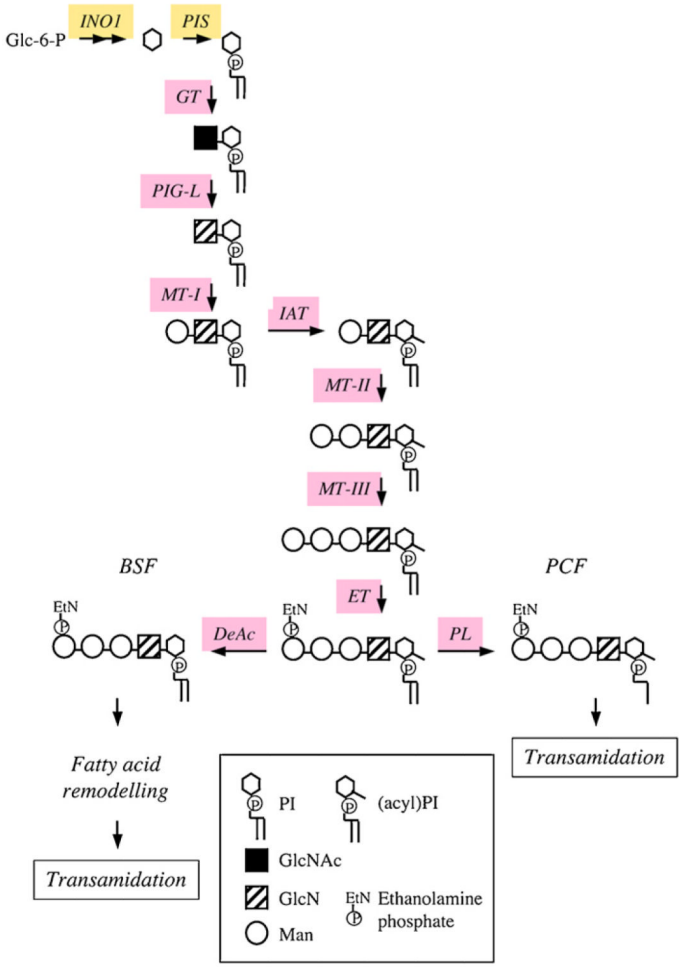

Fig. 4.

Pathway for GPI biosynthesis in *T. brucei*. Enzymes for which candidate genes have been identified in the *T. brucei* gene DB are indicated; see Table 1 for the abbreviations of the enzymes. BSF, bloodstream forms; PCF, procyclic forms.

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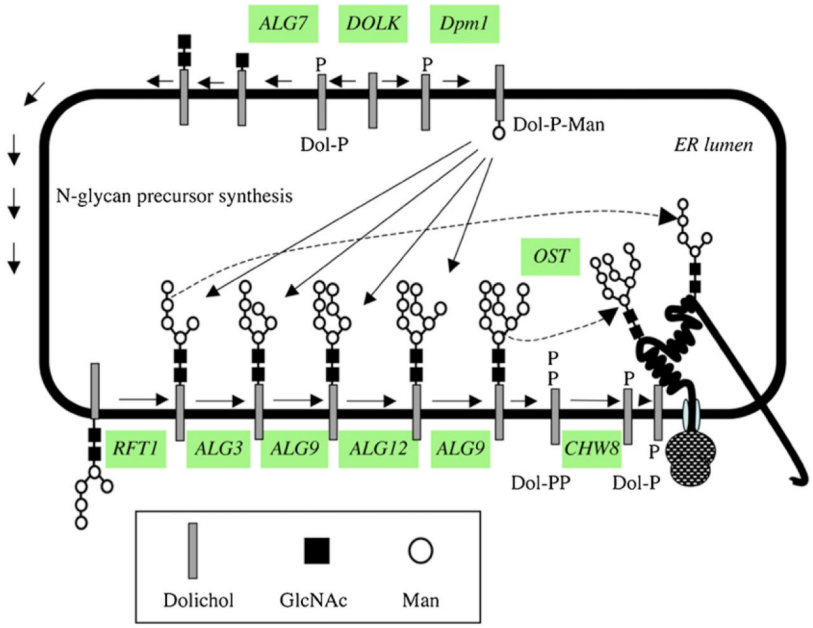

Fig. 5.

Predicted pathway for N-glycosylation in *T. brucei*. Enzymes for which candidate genes have been identified in the *T. brucei* gene DB are indicated; see Table 1 for the abbreviations of the enzymes.

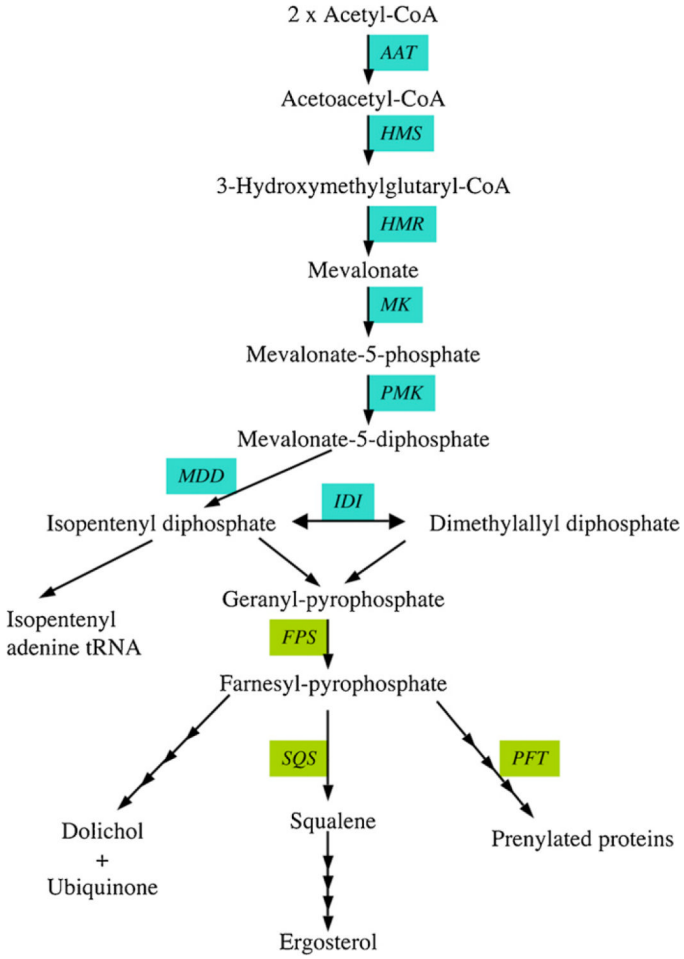

Fig. 6.

Predicted pathways for mevalonate and isoprenoid biosynthesis in *T. brucei*. Enzymes for which candidate genes have been identified in the *T. brucei* gene DB are indicated; see Table 1 for the abbreviations of the enzymes.

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Table 1

Trypanosoma brucei genes encoding predicted enzymes involved in membrane lipid biosynthesis.

Biosynthetic step	Enzyme name	*T. brucei* gene	Essential[a]	Yeast gene[a]
ELO1	Elongase 1	Tb927.7.4160	No (both)	
ELO2	Elongase 2	Tb927.7.4170	No (BSF)	
ELO3	Elongase 3	Tb927.7.4180	No (both)	
ELO4	Elongase 4	Tb927.5.4530	No (BSF)	ELO1-3
ACS1	Acyl-CoA synthetase 1	Tb09.160.2770		
ASC2	Acyl-CoA synthetase 2	Tb09.160.2780		FAA2
ASC3	Acyl-CoA synthetase 3	Tb09.160.2810		FAA1/FAA4
ASC4	Acyl-CoA synthetase 4	Tb09.160.2840		
ASC5	Acyl-CoA synthetase 5	Tb927.10.3260	No (both)	FAA2
ACBP	Acyl-CoA binding protein	Tb927.4.2010 Tb11.52.0001	BSF	ACB1
SPT	Serine palmitoyltransferase	Tb927.4.1020		LCB1/LCB2
3KSR	3-Ketosphinganine reductase	Tb927.10.4040		TSC10
DHCS	Dihydroceramide synthase	Tb927.8.7730 Tb927.4.4740		LAG1/LAC1
DHCD	Dihydroceramide desaturase	n.i.		SUR2/SYR2
SK	Sphingosine kinase	Tb927.7.1240		LCB4/LCB5
SPL	Sphingosine-1-phosphate lyase	Tb927.6.3630		DPL1
SLS1	Sphingolipid synthase 1	Tb09.211.1000		
SLS2	Sphingolipid synthase 2	Tb09.211.1010		
SLS3	Sphingolipid synthase 3	Tb09.211.1020		
SLS4	Sphingolipid synthase 4	Tb09.211.1030		AUR1
GAT1	Glycerol-3-phosphate acyltransferase	Tb927.10.3100		GAT1/2
GAT2	1-Acyl-*sn*-glycerol-3-phosphate acyltransferase	Tb11.01.6800		GAT1/2
DAT	Dihydroxyacetonephosphate acyltransferase	Tb927.4.3160		SLC1
ADS	1-Alkyl-dihydroxyacetonephosphate synthase	Tb927.6.1500		ADS1
PAP	Phosphatidic acid phosphatase	Tb927.6.1820		PAH1/LPP1
DAGK	DAG kinase	Tb927.8.5140		DGK1
DAGAT	DAG acyltransferase	Tb11.18.0008 Tb927.3.1700		DGA1
CK	Choline kinase 2	Tb11.18.0017	Both	CK1/2
CCT	Choline-phosphate cytidylyltransferase	Tb927.10.12810	PRO	CCT1
CPT	Choline phosphotransferase	Tb927.10.8900	PRO	CPT1
EK	Ethanolamine kinase 1	Tb927.5.1140	PRO	EK1/2
ECT	Ethanolamine-phosphate cytidylyltransferase	Tb11.01.5730	Both	ECT1
EPT	Ethanolamine phosphotransferase	Tb927.10.13290	Both	EPT1
CTPS	CTP synthase	Tb927.1.1240	BSF	URA7/8
CLS	Cardolipin synthase	Tb927.4.2560	PRO	CRD1
PGPS	Phosphatidylglycerophosphate synthase	Tb927.8.1720	PRO	PGPS
PSS/PSS2	PS synthase/PS synthase-2	Tb927.7.3760	Both	CHO1/PSS2

Biosynthetic step	Enzyme name	*T. brucei* gene	Essential[a]	Yeast gene[a]
PSD	Phosphatidylserine decarboxylase	Tb09.211.1610	BSF	PSD1/2
INO1	Inositol-3-phosphate synthase	Tb927.10.7110	BSF	INO1
PIS	Phosphatidylinositol synthase	Tb09.160.0530	Both	PIS1
PIKn	PI3 kinase Class (III)	Tb927.8.6210		
PIKn	PI4 kinase	Tb927.4.1140		PiK1
PIKn	PI4 kinase	Tb927.3.4020 Tb927.4.800 Tb927.4.420		Stt4
PIKn	PIK-related	Tb927.1.1930 Tb927.2.2260 Tb11.01.6300		
GPI-PLC	GPI phospholipase C	Tb927.2.6000	No (BSF)	
SMase	Neutral sphingomyelinase	Tb927.5.3710	Both	ISC1
PLA1	Phospholipase A_1	Tb927.1.4830	No (both)	N/A
LPLA1	Lyso-phospholipase A_1	Tb09.211.3650	No (both)	N/A
ALG7	UDP-GlcNAc:Dol-P GlcNAc-1-P transferase	Tb11.01.2220		ALG7
ALG1	GDP-Man:$GlcNAc_2$-PP-Dol β-1,4-mannosyltransferase	Tb927.10.13210		ALG1
ALG2	GDP-Man:Man_1 $GlcNAc_2$-PP-Dol α-1,3-mannosyltransferase	Tb927.4.2230		ALG2
ALG11	α-1,2-Mannosyltransferase	Tb09.211.0860		ALG11
ALG3	Dol-P-Man α-1,3-mannosyltransferase	Tb927.10.6530	No (BSF)	ALG3
ALG9	Dol-P-Man:$Man_5GlcNAc_2$-PP-Dol α-1,2-mannosyltransferase	Tb927.6.1140		ALG9
ALG12	Dol-P-Man:$Man_7GlcNAc_2$-PP-Dol α-1,6-mannosyltransferase	Tb927.2.4720		ALG12
OST	Subunit of the oligosaccharyltransferase complex	Tb927.5.890 Tb927.5.900 Tb927.5.910		STT3 (OST)
RFT1	$Man_5GlcNAc_2$-P-P-Dol translocase	Tb11.01.3540		RFT1
GDMPP	GDP-Man pyrophosphorylase	Tb927.8.2050	BSF	PSA1
DOLK	Dolichol kinase	Tb09.211.3740	Both	SEC59
DPMI	Dol-P-Man synthase	Tb927.10.4700	Both	DPM1
CHW8	Dol-PP phosphatase	Tb927.6.1820	BSF	CHW8/ CAX4
(GT)	GPl-GlcNAc transferase			
TbGPI13		Tb927.2.1780		GPI3
TbGPI12		Tb927.3.4570		GPI2
TbGPI15		Tb927.10.6140		GPI15
TbGPI19				GPI19
TbGPI1				GPI1 ERI1
(PIG-L) TbGPI2	GlcNAc-PI de-*N*-acetylase	Tb11.01.3900	BSF/ No(PRO)	GPI2
(MT-I) TbGPI14	α-1-4-Mannosyltransferase (MT-I)	Tb927.6.3300		GPI14 PBN1
(MT-II) TbGPI18	α-1-6-Mannosyltransferase (MT-II)	Tb927.10.13160		GPI18
(MT-III) TbGPI10	α-1-2-Mannosyltransferase (MT-III)	Tb927.10.5560	BSF	GPI10
IAT	Inositol acyltransferase	n.i.		GWT1

Biosynthetic step	Enzyme name	*T. brucei* gene	Essential[a]	Yeast gene[a]
(ET)	Ethanolamine-phosphate transferase			
TbGPI13		Tb11.02.2720		GPI13
TbGPI11		Tb927.10.13290		GPI11
DeAc	GPI inositol deacylase			
TbGPIdeAc, TbGPIdeAc2		 Tb927.3.2610	BSF	BST1
TbGUP1	GPI remodellase	Tb10.61.0380	No (BSF)	GUP1
Transamidase TbGPI8 TbGAA1 TbGPI16	GPI transamidase subunit 8 GPI transamidase subunit Gaa1 GPI transamidase subunit 16	Tb927.10.13860 Tb927.10.210 N/A	BSF/ No(PRO) BSF/ No(PRO)	GPI8 GAA1 GPI17
TTA1		Tb927.4.1920		GPI16
TTA2	GPI transamidase subunit Tta1 GPI transamidase subunit Tta2	N/A Tb11.01.7400 Tb927.10.5080		GAB1
AcCS	Acetyl-CoA synthetase	Tb927.8.2520		ACS1
AAT	Acetyl-CoA acetyltransferase	Tb927.8.2540		ERG10
HMS	HMG-CoA synthase	Tb927.4.2700		ERG13
HMR	HMG-CoA reductase	Tb927.6.4540		HMG1/2
MK	Mevalonate kinase	Tb927.4.4070		ERG12
PMK	Phosphomevalonate kinase	Tb09.160.3690		ERG8
MDD	Mevalonate-diphosphate decarboxylase	Tb927.10.13560	BSF	ERG19
IDI	Isopentenyl-diphosphate isomerase	Tb09.211.070	BSF	IDI
FPS	Farnesyl-pyrophosphate synthase	Tb927.7.3360	BSF	ERG20
PFT	Protein farnesyltransferase α-subunit Protein farnesyltransferase β-subunit	Tb927.3.4490 Tb927.7.460	BSF	RAM1
SQS	Squalene synthase	Tb927.8.7120		ERG9

n.i., no gene identified.

[a]Indicates if a gene product is essential for parasite growth in culture; BSF, bloodstream forms; PCF, procyclic forms; both, essential in both bloodstream and procyclic forms; no, not essential.

[b]The corresponding *S. cerevisiae* genes.