The metabolic repertoire of *Leishmania* and implications for drug discovery

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Abstract

The development of new anti-*Leishmania* compounds and the study of the mode of action of existing drugs has long been hampered by the difficulties of obtaining sufficient numbers of intracellular amastigotes or to culture in the laboratory well-defined parasites representative of intracellular amastigote stages. Most of the early studies have been carried out on promastigotes, of which the metabolism is not necessarily identical to that of amastigotes. Also reseach has been complicated by the fact that amastigotes hide away inside the phagosome of the host cell, the macrophage, which imposes additional diffusion barriers and the possibility of metabolizing or degrading drugs before they reach the target. Only a limited number of drugs is available for the treatment of leishmaniasis and hardly anything is known about their mode of action. The recent completion of the *Leishmania major* genome sequencing project now provides a more complete insight in the metabolic capacities of this and related parasites. Important differences between *Leishmania* and trypanosomes have been identified, and these differences could be related to the special adaptations required for the viability of *Leishmania* inside the macrophage's phagosomal compartment and for its virulence. Differences that exist between parasite and host could be exploited as potential targets for drugs with high selectivity. Despite the fact that glycolysis is not an essential pathway in the intracellular amastigote, its overall functionality is of vital importance, by the fact that many of its enzymes are involved in the reverse process of gluconeogenesis. Also enzymes responsible for mannan synthesis from either hexoses or gluconeogenic substrates are validated drug targets. Glucose transporters and fructose-bisphosphatase, as an exclusive gluconeogenic enzyme, have been validated as drug target. By virtue of the distinctive structural properties exhibited by the enzymes shared by the glycolytic and gluconeogenic pathways and the fact that the majority of them is ensconced inside glycosomes, organelles which require a complete machinery for their biosynthesis and for which the individual peroxin proteins differ considerably from their mammalian counterparts, both glycolytic enzymes and peroxins are interesting drug targets. The methylglyoxal bypass, although not yet validated as a target, certainly deserves further study. Folate transporters have not yet been exploited and three haem biosynthetic enzymes which are all conserved within *Leismania* and for which there is no obvious function, deserve further investigation. Other interesting drug targets are NADP-dependent fumarate reductase, acetate:succinate CoA transferase, dihydroorotate dehydrogenase and arginase. Finally genomic information has provided a possible explanation for the mode of action of the newly introduced anti-leishmanial drug Miltefosine.

Introduction

In this chapter we consider the impact that the completion of the *Leismania major* genome sequencing project has had upon our understanding of its metabolism and the possibilities that this knowledge provides for the development of new drugs against this medically relevant trypanosomatid. The different environments, in both the phagosomal compartment of the mammalian macrophage and its phlebotomine vector, that are occupied by *Leishmania major* means that a correct interpretation of the metabolic strategies used by the parasite in each of the hosts is particularly difficult. In comparison to several other protozoan parasites for which complete genome information is available, such as the sleeping sickness parasite *Trypanosoma brucei* (Berriman et al., 2005; Ginger et al., 2007) and the malarial parasite *Plasmodium falciparum* (Gardner et al., 2002), little is known about the actual metabolism of the pathogenic stage, the amastigote. Notwithstanding this fact, the availability of genome sequences, coupled to the use of modern molecular approaches to study gene function, have facilitated a significant reappraisal in our fundamental understanding of the metabolism in this organism.

Significant metabolic differences exist between *Leishmania* promastigotes and amastigotes. In the female sandfly promastigotes are adapted for life within the different parts of the fly's intestinal tract or the proboscis when they migrate there as metacyclics. Sandflies live on a complex diet. In addition to blood, they feed on nectar and aphid honeydew and thus promastigotes should be able to digest not only a variety of sugars but also amino acids. The intracellular amastigote thrives within a phagosome and phogolysosomes of mononuclear cells in a mammalian host. The infectious metacyclic promastigote in the proboscis of the sandfly must not only be capable of reaching this niche but, after its transformation to amastigote, should also be metabolically adapted to this more hostile environment. Apart from the fact that these compartments are acidic, little is known about the conditions prevalent in the phagosome, and this limits our ability to mimic these conditions experimentally. Nevertheless, some inferences could be made from the nature of the hydrolytic enzymes present in the phagosome and transporters in the phagosomal membrane (Burchmore and Barrett, 2001). The extensive information on the genes of *L. major* should give us a reasonably complete idea of the metabolic arsenal that is available to this parasite. However, at present it is far from clear in which stage of the life cycle the many gene products are operational and this hinders our understanding greatly.

Experimental studies of metabolism in *Leishmania* have focused mainly on the easily cultured promastigotes corresponding to the parasites found in the sandfly midgut. Other important life-cycle stages, such as metacyclic promastigotes and amastigote forms are more difficult to obtain and cannot be isolated easily in sufficient quantities for detailed biochemical studies. Metabolic studies have mainly concentrated on *L. donovani* and *L. mexicana*, with the underlying assumption that metabolic differences between the various species would be minimal. The study of amastigote metabolism originally has been complicated by the presence of the host cell. Although axenic amastigote model systems have more recently also become available, we have, until recently, had far less insight into the parasite's metabolic strategies that are important once it is in the mammalian

host. Despite the detailed metabolic maps that can be drawn from the genome-derived sequences it is in most cases impossible to distinguish between typical promastigote and amastigote adaptations. Thus, the *in silico* identification of potential drug targets should be complemented with further global transcriptomic and, in view of the post-transcriptional regulation of expression, particularly proteomic analyses (McNicoll et al., 2006) in order to complete our understanding of the adaptations and important metabolic switches that take place when the parasite invades the macrophage. It will be essential to experimentally demonstrate the functionality of a drug target in the pathogenic stage of the parasite. This should be followed by a (conditional) disruption of both alleles of the corresponding gene in order to demonstrate that the identified target plays an essential role in the survival, infectivity and/or virulence of the parasite. Our discussion focuses mainly on the identification of potential drug targets in *L. major*. When additional information about the target in the amastigote, or information from other trypanosomes, is available, these are discussed in a comparative context.

Drugs available for treatment of leishmaniasis

For the treatment of leishmaniasis the currently used drugs are limited to six. The most widely used first-line drugs available for all forms of leishmaniasis are pentavalent antimony preparations such as Pentostam (sodium stibogluconate) and Glucantime (N-methylglucamine antimonate). These drugs are administered parenterally for 10 to 30 days. Both formulations are equally effective but also equally toxic when efficacy is expressed in relation to the amount of antimony administered. The pentavalent antimony needs to be reduced to the trivalent state by either host or parasite metabolism in order to exert its activity (see chapter by Ouelette). Although antimony-based drugs have been used since 1947, their mode of action is still far from understood; they may disrupt the parasite's energy production or interfere with trypanothione metabolism. Unfortunately, in many parts of the world, the parasite has become resistant to antimony and failures and relapses occur in all forms of leishmaniasis and constitute approximately 10-25% of cases. If antimony-based drugs are not effective, the second line compounds of pentamidine (Lomidine) and amphotericine B (Fungizone) are used, which have been introduced in 1940 and 1959, respectively. The mode of action of pentamidine is not clear although there is some indication that it may act on the parasite's mitochondrion (Basselin et al., 2002; Muhkerjee et al., 2006). Amphotericin is a polyene antimycotic drug, which is believed to interact with membrane sterols, such as ergosterol present in *Leishmania*'s plasma membrane, to produce an aggregate that forms a transmembrane channel resulting in the loss of intracellular solutes and ions. Ambisome (introduced in 1994) is a formulation of amphotericine B in liposomes. Owing to the high capacity of cells of the reticuloendothelial system for phagocytosis the drug is specifically targeted and taken up by the host cells of the *Leishmania* parasite. This will increase efficacy and reduce toxicity of the drug. An important drawback of this formulation is its high cost. A new first line drug for the use of visceral leishmaniasis was introduced in 2002. It is the ether-lipid analogue Miltefosine (hexadecylphosphocholine, Impavido). This lysophospholipid, originally used for the treatment of certain types of cancer, has been shown to interfere with the synthesis of phospholipids and sterols in *Leishmania* (Lux et al, 2002; Rakotomanga et al, 2007). The advantage of this drug is that it is given orally

and is very effective. Miltefosine was originally tested for visceral leishmaniasis in India. The cure rate by Miltefosine is 95%. Studies in Ethiopia showed that it is also effective in Africa and clinical trials in Colombia demonstrated a high efficacy for cutaneous leishmaniasis as well. It is now registered in many countries and is the first orally administered breakthrough therapy for both visceral and cutaneous leishmaniasis. A side effect is gastrointestinal disturbance. However, Miltefosine resistance in laboratory strains of *Leishmania* has already been reported. Several other drugs, in particular the antifungal azoles itraconazole, ketoconazole, and fluconazole, have been on limited clinical trials. These drugs are supposed to act via inhibition of cytochrome P450, essential in the synthesis of ergosterol. So far, the results were equivocal. Allopurinol, a drug in use for the treatment of gout, probably functions as an alternative substrate for *Leishmania*'s hypoxanthine-guanine phosphoribosyl transferase, an enzyme located inside glycosomes. The allopurinol riboside produced is then incorporated into RNA causing inhibition of protein synthesis in the parasite. It has been used experimentally for human leishmaniasis, with variable degrees of success, and is also on trial for the treatment of Chagas' disease. This drug is now widely used for the treatment of leishmaniasis in dogs.

Despite the fact that several drugs are available for the treatment of leishmaniasis, new and better drugs are urgently required. Most available drugs are costly, require long treatment regimes and are becoming more and more ineffective.

The process of drug development

Only a very limited number of drugs have been developed for the treatment of leishmaniasis over the last sixty years and the efficacy of their use has been hampered by adverse effects or resistance of the parasite (see above). The greatest benefit of the genome sequencing project would be the ultimate development of new, effective and safe medicines for the treatment of all manifestations of leishmaniasis through the identification of one or more ideal drug targets. It has been the purpose of this review to present a comprehensive analysis of the major metabolic pathways of *L. major* with, where possible, an emphasis on amastigote-specific features that may be essential for the parasite's adaptation to the host. An important assumption made in this review is that metabolic differences between *L. major* and other species of *Leishmania* are minimal or non-existent. A preliminary comparison with the incomplete genomic information already available for *L. infantum* and *L. brasiliensis* seems to confirm this idea (Opperdoes and Coombs, 2007).

Obvious drug targets are those pathways that are absent from the host, or individual reactions that are catalysed by enzymes with structural properties that are distinct from host enzymes. In this respect, enzymes that are the results of events of lateral gene transfer from bacteria to a trypanosomatid ancestor are very interesting candidates. Unique pathways or enzymes should be validated first as drug targets by demonstrating, either by inhibition studies and through reverse genetic approaches, that they fulfill an essential role in the viability or virulence of the amastigote. Here the "*Leishmania* researcher" has a disadvantage with respect to his colleagues working with *T. brucei*.

RNA interference cannot be used and thus one has to take the laborious road of the creation of (conditional) gene disruption mutants. It should also be kept in mind that only results obtained with macrophages infected with amastigotes can be considered as relevant. Enzyme inhibitors to be selected as leads should be tailored in such a way that they will be able to reach a target hidden away inside the phagosome of the macrophage. Promising lead compounds should be effective not only in axenic amastigotes but also in *in vitro* studies with amastigotes that multiply in infected macrophages, before they should be used in an appropriate animal model. Finally, formulations should be developed that allow them to be administered *per os* in order to guarantee that such a drug will be available at low cost to the poor populations who are most in need of a new anti-leishmanial drug. Each of these steps towards the development of a new anti-leishmanial drug poses a formidable task with limited chances of success. Because of the low economic returns to be expected for private industry from a drug developed for the poorest populations of the world, the interest for the development of such drugs has always been limited and will certainly not increase in the future. With this chapter we try to facilitate one of the first steps in this process, the identification of new drug targets.

Outline of promastigote and amastigote metabolism

Biochemical analyses on *Leishmania* promastigotes have shown that these stages can use both glucose and amino acids, such as proline, as energy sources. The catabolism of these substrates appears to involve both glycolysis, compartmentalized in peroxisome-like organelles called glycosomes, and mitochondrial metabolism with an active tricarboxylic acid (TCA) cycle and linked electron transport chain. There is evidence that other sugars than glucose could also be used. This would especially be important in the case of midgut stages where plant sugars should be abundant. The presence of a glyoxylate cycle once reported, seems to be absent which would mean that fatty acids may not serve as the sole substrates for gluconeogenesis. In addition to carbon dioxide, other end-products of promastigote metabolism include succinate and smaller amounts of acetate, pyruvate, D-lactate, alanine, ammonia or urea. Considerably more fragmentary is our knowledge of the energy metabolism of amastigotes. The main reason for this is that these intracellular stages have been less available for study. The limited number of studies carried out show that *L. mexicana* amastigotes isolated from *in vivo* lesions have an increased beta-oxidation of fatty acids and a reduced need for proline and glucose consumption (Hart and Coombs, 1982). The full complement of TCA-cycle enzymes and respiratory chain are present. Glycosomes are less abundant in amastigotes than in promastigotes (Coombs et al., 1986; Tetley and Vickerman, 1991), but have a bigger arsenal of enzymes such as malate dehydrogenase and phosphoenolpyruvate carboxykinase (Mottram and Coombs, 1985a, 1985b). Results of recent studies carried out on *in vitro* cultured amastigotes largely agree with the findings with lesion-derived amastigotes, thus opening the opportunity for more extensive analyses of the energy metabolism of the infectious stages of these parasites.

Enzymes of carbohydrate catabolism and gluconeogenesis as drug target

From previous studies, it is well known that the *Leishmania* promastigote relies mainly on glycolysis and amino-acid metabolism for energy generation. Glucose and other hexose sugars, such as fructose and mannose, are converted to carbon dioxide, alanine, succinate and acetate which are excreted as the major end-products (Bringaud et al., 2006). The identification in the *L. major* genome of several enzymes involved in the metabolism of C5 sugars, such as ribokinase, ribulokinase and xylulokinase, suggests that this trypanosomatid is equally able to catabolise sugars, other than the hexoses, present in nectar and aphid honeydew, components of the sandfly diet. The genes for ribulokinase and xylulokinase seem to have been acquired by lateral gene transfer from a bacterium, since they have homologues only in prokaryotes. Promastigotes are also capable of hydrolyzing disaccharides through the secretion of disaccharide splitting enzymes (Blum and Opperdoes, 1994) and in agreement with this at least three bacterial-type genes annotated as sucrase (invertase) or beta-fructosidases have been found in the *L. major* genome. *Leishmania* also accumulates an intracellular storage product, β-mannan, a mannose-rich polysaccharide (Keegan and Blum, 1992, 1993; Ralton et al., 2003), thus some of these enzymes could also be involved in the degradation of this polysaccharide as well. Owing to the unique nature of these enzymes and because mannan degradation seems to be important for amastigote virulence, they would be interesting drug targets, as discussed below.

The activities of many enzymes of carbohydrate metabolism (Table 1) in both *L. mexicana* cultured promastigotes and amastigotes isolated from lesions in mice have been described (Coombs et al., 1982; Mottram and Coombs, 1985a and b). These activities involve several glycolytic enzymes, fructose-1,6-bisphosphatase, a key enzyme of gluconeogenesis, malate dehydrogenase and phosphoenolpyruvate carboxykinase, and glucose-6-phosphate dehydrogenase, the first committed enzyme of the pentose-phosphate pathway. The specific activities of some glycolytic enzymes (hexokinase, phosphofructokinase) and glucose-6-phosphate dehydrogenase were somewhat lower in amastigotes, while that of pyruvate kinase was drastically reduced. In contrast, the activities of malate dehydrogenase, phosphoenolpyruvate carboxykinase and glucose-6-phosphate isomerase were greatly increased. The subcellular organization of carbohydrate metabolism in *Leishmania* parasites is similar to that in procyclic *T. brucei*, with the major part of the glycolytic pathway, malate dehydrogenase and phosphoenolpyruvate carboxykinase in glycosomes, and a dual distribution of enzymes of the pentosephosphate pathway (PPP) (Coombs et al., 1982; Mottram and Coombs, 1985a and b; Hart and Opperdoes, 1984; Maugeri et al., 2003; Veitch et al., 2004). All of the genes encoding proteins necessary for the uptake and degradation of glucose via the glycolytic pathway were already identified prior to the arrival of the *Leishmania* genome sequences (reviewed by Opperdoes and Michels, 2001 and Michels et al., 2006). The C5-sugar kinases carry a peroxisome-targeting signal (PTS) and thus are most likely present inside glycosomes, as well and they allow the C5 sugars to feed into the glycolytic pathway via the PPP enzymes.

Gluconeogenesis

While promastogotes feed mainly on sugars, the amastigote stage in the phagosome feeds probably mainly on fatty acids and amino acids and may have limited access to

glucose and other sugars generated from glycosylated proteins and glycolipids being degraded in the phagosomal compartment. Sugar residues required by *Leishmania* for protein glycosylation, glycophospholipid-anchor formation and polysaccharide biosynthesis thus have to be formed *de novo* from oxaloacetate via the gluconeogenic pathway (Fig. 1). The enzymes essential for gluconeogenesis (i.e. besides many glycolytic enzymes also phospho*enol*pyruvate carboxykinase and fructose-1,6-bisphosphatase), as well as the genes involved in glycosylation and polysaccharide formation have all been identified and some carry PTSs. Also genes for two enzymes involved in the antagonistic regulation of both glycolysis and gluconeogenesis, 6-phosphofructo-2-kinase and fructose-2,6-bisphosphatase, are present (Chevalier et al., 2005). These two enzymes are responsible for the formation and degradation of fructose-2,6-bisphosphate, which in many organisms is an allosteric regulator of phosphofructokinase. However, in trypanosomatids this compound regulates the activity of the cytosolic pyruvate kinase (Van Schaftingen et al., 1985). In agreement with the cytosolic function of this regulator these two enzymes lack a canonical PTS and have been shown to be cytosolic (Van Schaftingen et al., 1985).

The capacity of glucose uptake of amastigotes is considerably less than that of promastigotes (Burchmore and Hart, 1995). Uptake is mediated via three glucose transporters encoded by homologous genes (LmGT1-3). In the case of *L. mexicana* LmGT1 and LmGT3, having different substrate affinities, are constitutively expressed in, respectively, the flagellar and the plasma membrane, while LmGT2 is a high affinity transporter that is only expressed in the plasma membrane of the promastigote. Thus each glucose transporter isoform has a distinct biological function in the parasite. (Burchmore and Landfear, 1998). These transporters are capable of importing a variety of hexoses (glucose, mannose, fructose, galactose) into the cells (Rodriguez-Contreras et al., 2007). Thus also the amastigote is capable of catabolizing hexose sugars via the glycolytic and pentose-phosphate pathways although at a reduced rate. Because of the apparent low pyruvate kinase activity in the intracellular stage, glucose degradation does not necessarily result in the production of pyruvate, but rather succinate via the phospho*enol*pyruvate carboxykinase/malate dehydrogenase branch in the glycosome, which also comprises fumarate hydratase and NADH-dependent fumarate reductase. This enzyme which is involved in an essential pathway of glycosomal NADH reoxidation, is not found in higher eukaryotes and thus could be an interesting drug target. Indeed a number of inhibitors of fumarate reductase have been shown to exert potent anti-leishmanial activity (Chen et al, 2001). Succinate can also be produced by mitochondrial metabolism, very similar as has been demonstrated for procyclic *T. brucei* (Bringaud et al., 2006; Michels et al., 2006). Another end-product, alanine, is produced via the transamination of pyruvate by alanine aminotransferase, an enzyme which is expressed in both life-cycle stages (Mottram and Coombs, 1985b). Although hexoses are probably less abundant in the phagosomes than other nutrients such as amino acids or lipids, monosaccharides too may be made available to amastigotes by the hydrolytic activity of phagosomal glucuronidase on glycosylated proteins delivered by the variety of vesicles fusing with the phagosome.

Recent research has provided another, possibly more important role for the glucose transporters and various glycolytic enzymes in amastigotes. Both infectious metacyclic

promastigotes and amastigotes synthesize large amounts of β-mannan, that was shown to be essential for amastigote replication and thus virulence (Ralton et al., 2003). Glucose-transporter null mutants of *L. mexicana* are still viable as promastigotes because they can still grow on other nutrients than glucose, but amastigotes don't (Rodriguez-Contreras et al., 2007; Burchmore et al., 2003; Rodriguez-Contreras and Landfear, 2006); macrophage infectivity is not affected, but the intracellular parasites don't replicate. Mutant promastigotes synthesize carbohydrate-containing macromolecules from substrates such as alanine, glycerol, aspartate and acetate, but particularly the synthesis of β-mannan is significantly reduced (Rodriguez-Contreras and Landfear, 2006). This synthesis can be accounted for by gluconeogenesis, but the data suggest that this process cannot fully compensate for the reduced β-mannan synthesis resulting from the loss of hexose uptake. Indeed, a null mutant without the gluceneogenic enzyme fructose-1,6-bisphosphatase could grow as promastigotes on hexose-containing medium and synthesize β-mannan, from the hexoses, but not produce this oligosaccharide from gluconeogenic substrates (Naderer et al., 2006). The mutant promastigotes were internalized by macrophages but were unable to replicate in the phagosome and generate lesions in mice. These data validate enzymes responsible for mannan synthesis from either hexoses or gluconeogenic substrates as drug targets. These involve the hexose transporters, hexokinase, glucose-6-phoshate isomerase, fructose-1,6-bisphosphatase and enzymes involved in β-mannan synthesis from fructose 6-phosphate, such as mannose-6-phosphate isomerase, phosphomannomutase and GDP-mannose pyrophophorylase, but probably also a variety of other glycolytic enzymes acting in the gluconeogenic direction (aldolase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglycerate mutase, enolase, phosphoenolpyruvate carboxykinase, malate dehydrogenase and glycerol-3-phosphate dehydrogenase). Which enzyme would be the best drug target will depend on the substrates available inside the phagosome and this has to be established first. For several of the enzymes of *Leishmania* carbohydrate metabolism, of their homologues from *T. brucei* or *T. cruzi*, crystal structures have already been determined and selective inhibitors developed. For some of them it has been shown that they kill cultured parasites (reviewed in: Verlinde et al, 2001; Opperdoes and Michels, 2001).

Methylglyoxal bypass

Leishmania has been reported to produce **D**-lactate (Darling and Blum, 1988), an end-product of methylglyoxal metabolism (Fig. 2). In bacteria, under conditions of phosphate starvation, methylglyoxal is formed from triosephosphates by means of a methylglyoxal synthase. However, a gene encoding such an enzyme appears to be absent from *Leishmania*, as well as from the other trypanosomatids (Table 2). Nevertheless, methylglyoxal can also be formed by fragmentation of triosephosphates through a spontaneous reaction or via a side reaction of the enzyme triosephosphate isomerase. Methylglyoxal is converted to **D**-lactate by a thiol-dependent glyoxalase system. Interestingly the trypanosomatid glyoxalases I and II use trypanothione, rather than glutathione, as the essential cofactor (Irsch and Krauth-Siegel, 2004). Both enzymes are present in *L. major*, in agreement with the production of **D**-lactate by this organism. Glyoxalase I seems to have prokaryotic ancestry (Vickers et al., 2004). *L. brasiliensis*, however, seems to lack the glyoxalase II gene, which would suggest that also this

organism would be unable to form **D**-lactate. *T. brucei*, which has a much more active glycolysis and therefore is thought to produce more methylglyoxal, lacks the glyoxylase I gene and does not form any **D**-lactate. Methylglyoxal is thought to be formed mainly inside glycosomes, the site of the first part of the glycolytic pathway, but the two enzymes of the glyoxalase system do not carry an identifiable PTS.

Interestingly, in contrast to *Trypanosoma* species, *Leishmania* possesses a **D**-lactate dehydrogenase. This enzyme may convert the **D**-lactate into pyruvate for further metabolism. The **D**-lactate dehydrogenases of three *Leismania* species (*L. major, L. infantum* and *L. brasiliensis*) have a PTS, which supports the idea that the glyoxalase pathway is also glycosomal. The fact that methylglyoxal is toxic and mutagenic, that the methylglyoxalase system of *Leishmania* significantly differs from that of humans, and that **D**-lactate dehydrogenase is absent from humans, makes this pathway an interesting drug target (Ariza et al., 2006).

Glycosome biogenesis

The unique sequestering of glycolysis and other pathways of carbohydrate metabolism in glycosomes in trypanosomatids and the vital importance of these processes to these parasites have led to the notion that this metabolic compartmentation itself might be essential as well. *T. brucei* homologues of twelve proteins, designated 'peroxins (acronym 'PEXs'), involved in biogenesis of peroxisomes of mammalian cells or yeasts, have been identified so far (reviewed in Moyersoen et al., 2004). Indeed, depletion of each of them by RNAi affected the proper localization of glycosomal enzymes and led to death of bloodstream-form trypanosomes and, at growth conditions requiring glycolytic activity, of procyclic forms too (Moyersoen et al., 2004; Furuya et al., 2002). The importance of proper compartmentation has been attributed to the specific kinetic features and lack of activity regulation of the trypanosomatid glycolytic enzymes; as a result, the glycolytic flux can only be controlled under the conditions pertaining inside the organelle, not under those of the cytosol (Bakker et al, 1997).

For *Leishmania*, the same 12 peroxin homologues have been identified in the genome, but only three of them (PEX2, PEX5, PEX14) have been experimentally analyzed (Table 3). The essentiality of two peroxins (PEX2 and PEX5) has been confirmed for *L. donovani* promastigotes (Flashpohler et al., 1997, 1999; Jardim, et al., 2000). The fact that several glycosomally-located enzymes of glycolysis and gluconeogenesis are involved in β-mannan synthesis, a process that is vital to amastigotes (see section on gluconeogenesis), strongly suggests that glycosome biogenesis will also be essential to this human disease-causing stage of the parasites and thus a candidate drug target.

The peroxins responsible for different aspects of glycosome biogenesis in trypanosomatids and the homologous peroxins involved in peroxisome biogenesis in humans are poorly conserved. The amino-acid sequences of those peroxins that have been identified have overall identities varying between 12 and 34% (Moyersoen et al., 2004), offering great perspectives for the discovery of compounds that specifically affect the function of the trypanosomatid proteins but not the human ones. Indeed, current research attempts to solve three-dimensional structures of trypanosomatid peroxins that interact with each other in the cascade of reactions leading to the organelle's synthesis,

and to develop compounds that prevent such interactions (Moyersoen et al., 2004, Choe et al., 2003).

Mitochondrial metabolism

The glycolytic end-product pyruvate is further metabolised in the mitochondrion to acetyl-CoA. A large part of the acetyl-CoA is converted to acetate by the TCA-cycle enzyme succinyl-CoA ligase and a unique acetate:succinate CoA transferase (ASCT) (Van Hellemond et al., 1998; Riviere et al., 2004). Together, the latter two enzymes catalyse a cycle leading to the net production one mole of ATP per mole of acetate produced (Van Hellemond et al., 1998; Bochud-Allemann & Schneider, 2002). Outside of the Trypanosomatidae, ASCT activity has only ever been found in certain kinds of anaerobic mitochondria, including hydrogenosomes (Tielens et al., 2002). The fact that this enzyme is absent from the mammalian host and is involved in ATP generation would render it an interesting drug target. The remainder of the acetyl-CoA can be metabolised via the enzymes of the TCA cycle, which are all present. An active respiratory chain comprising complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex III (cytochrome b/c oxidoreductase), cytochrome c and complex IV (cytochrome oxidase), is present and takes care of the reoxidation of mitochondrially produced NADH and succinate and the generation of a proton gradient over the mitochondrial inner membrane. ATP synthase is also present. It utilizes the proton gradient for the synthesis of ATP. Complex I, although larger than in *E. coli* (14 subunits), seems to be rather small. Only 18 NADH dehydrogenase subunits could be identified in the genome database against some 46 for mammalian Complex I (Opperdoes and Michels unpublished). No evidence was found for the involvement of this complex in the generation of the proton gradient, because a number of the integral membrane subunits supposed to be involved in proton translocation could not be identified. Delta-1-pyrroline-5-carboxylate dehydrogenase, which receives its reducing equivalents directly from proline, and electron transfer flavoprotein (ETF)-ubiquinone oxidoreductase, which accepts reducing equivalents through ETF from long, medium and short/branched chain fatty acid-specific mitochondrial acyl-CoA dehydrogenases, and which were reported recently for the trypanosomatids (Allen et al., 2004; van Weelden et al., 2005; Ginger et al., 2007) have also been identified in *L. major*.

Even though the presence of a complete set of TCA cycle enzymes can be predicted from the *Leishmania* genome sequence, they are apparently not used for the complete oxidation of acetyl-CoA to CO_2 as would occur if all enzymes are fully operational within the cycle. Indeed the fact that the mitochondrial isocitrate dehydrogenase utilizes NADP rather than NAD as cofactor suggests that this enzyme may have a function other than in the TCA cycle. This situation is reminiscent of what has been shown for the procyclic stage of *T. brucei* (Besteiro et al., 2002; van Weelden et al., 2003; van Weelden et al., 2005), where the primary role of the TCA cycle in the midgut stage parasite is the provision of precursors for biosynthetic pathways, such as gluconeogenesis, fatty acid synthesis and the oxidation of amino acids such as threonine and proline. Also in *Leishmania* promastigotes this seems to be the most important function of the TCA-cycle enzymes, as suggested by the large amounts of succinate and acetate produced under aerobic conditions. Since the amastigote is also actively involved in gluconeogenesis it is

not expected that mitochondrial metabolism here will be fundamentally different from what is encountered in procyclic *T. brucei*.

(i) Lack of an alternative oxidase

An unusual and key feature of the energy metabolism of bloodstream-form *T. brucei* is the mitochondrially-located alternative terminal oxidase which is involved in the reoxidation of NADH, produced in glycolysis, via a dihydroxyacetone-phosphate:glycerol-3-phosphate shuttle. A homologue of this oxidase was not found in *L. major*. Yet, *L. major* has in its mitochondrial membrane the FAD-dependent glycerol-3-phosphate dehydrogenase, which in trypanosomes is also part of the shuttle, but in *Leishmania* amastigotes most likely functions exclusively in the catabolism or use for gluconeogenesis of glycerol 3-phosphate derived from triglycerides being degraded in the phagosome (Guerra et al., 2006).

(ii) Iron-sulphur synthesis

Iron–sulphur clusters form redox prosthetic groups for a wide range of proteins, many of which are localised to the mitochondrion. The *L. major* genome appears to encode enzymes required for the synthesis of these molecules. There are a cysteine desulphurase which may be targeted to the mitochondrion and a mitochondrial iron-sulphur cluster NifU-like protein, suggesting that the organelle is indeed involved in the assembly of Fe–S cluster proteins using elemental sulphur.

(iii) Mitochondrial transporters

On the basis of genome analysis, *L. major* possesses a complete set of homologues of mammalian and yeast mitochondrial solute carriers. These are required for the transport of pyruvate, the end-product of glycolysis, and inorganic phosphate, and for the exchange of dicarboxylates and tricarboxylates. Without appropriate biochemical analysis it is not possible to determine the substrate specificities of individual carrier proteins on the basis of sequence homology alone, but the dicarboxylate and tricarboxylate carriers are probably involved in transport of TCA cycle intermediates and of aspartate and glutamate across the mitochondrial membrane. An ATP/ADP exchanger for the export of newly synthesized ATP from the mitochondrial matrix into the cytoplasm is also present.

Cofactor synthesis

Many enzymes use cofactors such as biotin, coenzyme A, FMN and FAD, NAD and NADP, pyridoxal phosphate and thiamine pyrophosphate. Humans obtain precursors of them as vitamins, required in small amounts in their diet, many other mostly microorganisms synthesize them *do novo*. The trypanosomatid genome database did not reveal the presence of any of the pathways involved in the *de novo* synthesis of these cofactors. However, trypanosomatid genome searches suggested that these parasites contain many of the enzymes involved in the final stages of the cofactors synthesis as known from other organisms, although the situation for biotin and thiamine is unclear

(Table 4). In contrast to the human situation, in which the last two enzymes of CoA biosynthesis have been fused to form a bifunctional enzyme, in *Leishmania* and the trypanosomes these are separate enzymes and, moreover, the last enzyme, dephospho-CoA kinase, is apparently of bacterial origin. Also the ATP-NAD kinase, responsible for the formation of NADP from NAD, seems of bacterial origin. This is an interesting aspect of *Leishmania* metabolism that deserves further study. Also specific transporters for precursors of cofactors should be interesting drug targets, but so far they have not yet been identified.

Folate metabolism

While in many other microorganisms such as bacteria and *Plasmodium* the folate pathway has proven to be a valuable target for drug intervention, no compounds targeting enzymes of folate metabolism have been found to be effective drugs against *Leishmania* infections (reviewed in Nare et al., 1997; Ouellette et al., 2002). There appear to be several reasons for this. Firstly, *Leishmania* are auxotrophes for folates (folate and biopterin) and must import these metabolites from an exogenous source (Nare et al., 1997; Ouellette et al., 2002). Consistent with this, no genes encoding enzymes of folate biosynthesis are present in the *L. major* genome, whereas there are as many as 12 genes encoding a novel class of transport membrane proteins responsible for folate transport (Richard et al., 2002; Cunningham and Beverley, 2001). Such transporters were previously only known to occur in cyanobacteria and in plant plastids (Klaus et al., 2005). Secondly, the enzymatic reduction of folate to become active as tetrahydrofolate, a coenzyme required for one carbon (C1) transfer reactions, can be catalysed by both the bifunctional dihydrofolate reductase-thymidylate synthase (DHFR-TS) (Beverley et al., 1986) and pteridine reductase 1 (PTR1). The latter is readily over-expressed if DHFR-TS is inhibited, thus it may be necessary to block both DHFR-TS and PTR1 simultaneously for effective interference with folate metabolism. *Leishmania* contains a complete set of genes for the activation and interconversion of C1 units (Table 4). For a full account of this metabolism the reader is referred to Opperdoes and Coombs (2007).

Requirement of haem

Haem is required for the synthesis of haemoproteins, such as the cytochromes *a, b, c* and P450. Endosymbiont-containing trypanosomatids are able to synthesize their own haem, but all other trypanosomatids, including *Leishmania,* are auxotrophes for haem which either has to be added to the culture medium in the form of haemin or as a component of the serum required for growth. In eukaryotes haem synthesis starts from succinyl CoA produced by the mitochondrial TCA cycle and includes a total of seven enzymatic steps of which the first and the last three take place in mitochondria. The other steps occur in the cytosol. In agreement with the haemin requirement of trypanosomatids the genes encoding the first four enzymes in the haem biosynthetic pathway, aminolevulinate synthase and aminolevulinate dehydratase (also known as porphobilinogen synthase) uroporphyrinogen-III synthase and uroporphyrinogen-III decarboxylase, have been detected neither in *Leishmania* (Sah et al., 2002) nor in other trypanosomatids. Protoporphyrin IX may substitute for haemin to grow *Leishmania* in

chemically defined medium, suggestive of ferrochelatase functionality in these cells (Chang and Chang, 1985). Indeed a ferrochelatase gene is present. However, the discovery, in three different species of *Leishmania,* of two genes homologous to coproporphyrinogen III oxidase and protoporphyrinogen oxidase, preceeding ferrochelatase in the pathway of haem biosynthesis, has come as a surprise. Moreover, these typical mitochondrial enzymes have no detectable mitochondrial transit peptide. These two oxidase genes, together with a pteridine transporter gene, are juxtaposed as a separate transcription unit at one end of chromosome 6 and, together with the ferrochelatase gene, seem all to be of bacterial origin. Does this mean that *Leishmania* has acquired by horizontal transfer the ability to synthesise haem from precursors either in the sandfly midgut or in the phagosome ? The macrophage has as one of its functions the removal from the circulation of erythrocytes that are at the end of their life time. Their subsequent degradation inside the phagolysosomes leads to the massive release of haem. Although haem degradation in mammalian cells does not include the production of coproporphyrinogen or protoporphyrinogen, maybe in the acidic environment of the lysosome some of the heam becomes chemically modified, escapes degradation to biliverdin and bilirubin and can be captured by the amastigote which then would require these three enzymes for its repair. It could be imagined that the acquisition of the three haem biosynthesis enzymes of bacterial origin has aided *Leishmania* to adapt to the extreme conditions prevailing in the macrophage phagolysosome (see also the section on oxidant stress protection below). Little is known about the structure of the haem incorporated into the *Leishmania* cytochromes other than that cytochrome c_1 has an atypical difference absorption spectrum and seems to be attached to the protein backbone via one, rather than two thioester linkages (Hill and White, 1968 ; Priest and Hajduk, 1992). Haem structure and synthesis in *Leishmania* certainly deserves further study.

Lipid metabolism

(i) *Beta* oxidation

Genome analysis suggests that *L. major* should be capable of oxidising fatty acids via *beta*-oxidation and this in two separate cellular compartments: the glycosomes and the mitochondrion (Table 5). Four homologues of the fatty acyl-CoA synthetases already identified for *T. brucei* (Jiang and Englund, 2001) are also present in *L. major*, each of them with a different chain-length specificity. Although no fatty acyl-CoA oxidase or dehydrogenase homologue with a PTS was found in the database, genes for the other enzymes involved in the *beta*-oxidation spiral, such as a bifunctional enzyme (with enoyl-CoA hydratase/enoyl-CoA isomerase/3-hydroxyacyl-CoA dehydrogenase activities, LmjF33.2600) and a 3-ketoacyl-CoA thiolase (LmjF23.0690), both having a PTS, were identified (Opperdoes and Szikora, 2006). Also four acyl-CoA dehydrogenases with specificity for, respectively, very long, long, intermediate and short/branched fatty acids were found in the genome. Three of them have a predicted mitochondrial transit peptide. Genes encoding a mitochondrial bifunctional enzyme and additional mitochondrial thiolases were also identified indicating that the mitochondrial compartment is also involved in beta-oxidation. The presence of genes coding for a 3,2-trans-enoyl-CoA isomerase and 2,4 dienoyl-CoA reductase suggests that *L. major* is also able to cope with unsaturated fatty acids. This resembles the situation found in mammals

where the peroxisomes are involved in oxidation of long chain fatty acids which, after shortening, are further degraded in the mitochondrion to acetyl-CoA. Apparently *L. major* is able to oxidise fatty acids of a wide variety in length and composition, but how each of the two compartments contribute to the oxidation of these fatty acids is far from clear and requires further experimentation. An additional argument for the involvement of the glycosome in lipid metabolism is the fact that in each of the three trypanosomatid genomes genes for three so-called half ABC transporters (GAT1-3) have been identified homologous to the peroxisomal transporters involved in fatty-acid transport. Indeed, for *T. brucei* it was confirmed that these transporters are associated with the glycosomal membrane (Yernaux et al., 2006). Preliminary data suggest that these transporters are involved in fatty acid transport over the glycosomal membrane.

(ii) Fatty acid synthesis

In addition to *beta*-oxidation of fatty acids, *L. major* is capable of fatty-acid biosynthesis. Mitochondrially produced citrate can be exported to the cytosol where it is cleaved by a citrate lyase into oxaloacetate and acetyl CoA. The latter can be converted by acetyl-CoA carboxylase to malonyl CoA for elongation in a novel type of fatty-acid biosynthesis pathway. A combination of comparative genomics and biochemical studies has revealed that the pathway used by trypanosomatids for fatty acid synthesis is most unusual (Lee et al., 2006; 2007; Table 5). There is no evidence for the presence of a cytosolic type-I fatty acid synthase, which is a multifunctional enzyme used for fatty-acid biosynthesis in many eukaryotes including mammals. Instead, three elongase genes seem to be responsible for the *de novo* synthesis of fatty acids in *T. brucei* (Lee et al., 2006; 2007). In mammals and yeast, elongases function to extend, via CoA-linked intermediates in a stepwise fashion, the end product (usually palmitate or stearate) of cytosolic type-I fatty acid synthesis, but in *T. brucei* these elongases, which have an overlapping specificity, function in extending butyryl-CoA stepwise to myristate or stearate (Paul et al., 2001). A similar set of elongases is present in the *L. major* genome, except that *L. major* has 12 tandemly linked homologous genes, rather than four, which may explain why in *Leishmania* much longer fatty acids can be synthesized. Despite its uniqueness in nature it remains to be seen whether in *Leishmania* this pathway of fatty-acid synthesis could function as a drug target. As will be discussed below, the oxidation of fatty acids is an import process in the amastigote and therefore fatty acid synthesis is expected to play a minor role in the metabolism of this pathogenic stage.

A type-II fatty acid synthesis with an acetyl-CoA carboxylase, acyl carrier protein (ACP), a keto-acyl (ACP) synthase, a keto-acyl (ACP) reductase and *trans*-2-enoyl (ACP) reductase (Van Weelden et al., 2005) is also present in *Leishmania*. The latter gene predicts a mitochondrial transit peptide, which suggests that the *Leishmania* mitochondrion contains a separate pathway for the synthesis of fatty acids, as has been shown in the case of *T. brucei.* Most likely this pathway is used for the synthesis of lipoic acid, an essential cofactor of the mitochondrial enzymes pyruvate dehydrogenase and 2-ketoglutarate dehydrogenase.

In addition, *L. major* synthesizes polyunsaturated fatty acids by using Delta6, Delta5 and Delta4 desaturases, and elongases specific for C18 Delta6 and C20 Delta5 polyunsaturated fatty acids. This suggests that a complete pathway for polyunsaturated

fatty acid biosynthesis is also present (Livore et al. 2007). Whether this pathway operates in the amastigote is not known.

(iii) Lipids as carbon sources for energy generation and biosynthetic pathways

L. mexicana amastigotes have been shown to contain higher activities than promastigotes of the enzymes that catalyse the beta-oxidation of fatty acids. This suggests that *beta*-oxidation of fatty acids is relatively more important to intracellular amastigotes living in a glucose-poor environment than promastigotes (Hart and Coombs, 1982; Coombs et al., 1982). A comparison of several pathogens, including *Leishmania*, that occupy phagosomal compartments inside mammalian macrophages (Lorenz & Fink, 2002; Lorenz et al., 2004; Munoz-Elias & McKinney, 2005) suggests that for organisms thriving in such a seemingly rather inhospitable niche, fatty-acid oxidation is critical for viability and virulence. Genome analysis shows that, in comparison with that of the other trypanosomatids, the *Leishmania* genome is particularly rich in genes involved in lipid metabolism, probably allowing it to degrade a wide variety of fatty acids. Acetyl-CoA, the end-product of beta-oxidation, is in general oxidised to carbon dioxide and water by the TCA cycle. There are some doubts as to whether the cycle functions as a cycle (see above). Acetyl-CoA can also be converted to acetate with the net synthesis of one molecule of ATP, via the succinyl-CoA synthase/ acetate-succinate CoA transferase cycle. The *L. major* genome has not revealed the presence of a functional glyoxylate cycle. This suggests that *Leishmania* would be unable to form sugars from fatty acids as sole carbon source and requires in addition an oxaloacetate-forming amino acids. After condensation of acetyl-CoA with oxaloacetate, the resulting citrate can be transported to the cytoplasm for the use in other reactions.

(iv) Synthesis of isoprenoids

Major components of cellular membranes in eukaryotic cells include sterols and phospholipids. Metabolic routes leading to the formation of sterols and other isoprenoids in *Leishmania* have been the subject of many biochemical studies that predated the availability of the trypanosomatid genome sequences and have been reviewed in detail elsewhere (Urbina, 1997). The availability of genome sequences now provides a fast track for cloning enzymes from an isoprenoid biosynthetic pathway that has considerable potential as a target for new trypanocidal drugs.

(v) Synthesis of phospholipids

The first steps in the synthesis of phosphatidic acid, the intermediate common to triacylglycerol and glycerophospholipids biosynthesis, take place inside glycosomes. Glycerol 3-phosphate (G3P) is either formed by reduction of dihydroxyacetone-phosphate (DHAP), catalysed by a glycosomal glycerol-3-phosphate dehydrogenase (G3PDH), or by the phosphorylation of glycerol using a glycosomal glycerol kinase. Subsequently, either G3P or DHAP undergo an initial acylation reaction that uses a fatty acyl-CoA molecule as acyl group donor. Only one gene encoding an appropriate putative acyltransferase, glycosomal DHAP acyltransferase, was detected in the *L. major* genome

(Table 6). This enzyme, which previously has been shown to be involved in the synthesis of ether lipids too (Heise and Opperdoes, 1997), resembles in many ways another, homologous G3P acyltransferase that is present in other organisms. However, no gene encoding an enzyme orthologous to the latter type of G3P acyltransferase was found in any trypanosomatid genome. Thus, it cannot be excluded that the glycosomal acyltransferase (LmjF34.1090) identified here has a wide substrate specificity accepting both DHAP and G3P as acyl acceptor. The second acyltransferase reaction is catalysed by lysophospholipid acyltransferase, or 1-acyl-sn-glycerol-3-phosphate acyltransferase. This enzyme, however, lacks a typical PTS and therefore one might assume that reactions of phospholipid assembly subsequent to that catalysed by DHAP acyltransferase probably do not occur in glycosomes. The formation of triacylglycerols and neutral phospholipids from phosphatidic acids begins with dephosphorylation, catalysed by a phosphatidate phosphatase. The resulting diacylglycerol can be directly acetylated to form a triacylglycerol by an acyltransferase or can react with CDP-choline or CDP-ethanolamine to form phosphatidylcholine or phosphatidylethanolamine, respectively. Ethanolamine-phosphate cytidylyltransferase and cholinephosphate cytidylyltransferase homologues were identified. In most organisms phosphatidylcholine can also be synthesised from phosphatidylethanolamine by a series of methylation reactions initiated by the enzyme phosphatidylethanolamine N-methyltransferase and this enzyme was detected in *L. major*, but not in *T. brucei* and *T. cruzi*. Finally, acidic phospholipids can be derived from phosphatidic acids after their reaction with CTP to form CDP-diacylglycerols. Both phosphatidylserine and phosphatidylinositol can be formed from the latter compounds since the corresponding phosphatidylserine and phosphatidylinositol synthases are present. Phosphatidylserine can be converted to phosphatidylethanolamine by a phosphatidylserine decarboxylase. The latter enzyme may be mitochondrial because it carries a predicted mitochondrial transit peptide. (Table 6).

Miltefosin (hexadecylphosphocholine, Impavido) is a lysophospholipid, which has been shown to interfere with the synthesis of phospholipids and sterols in both mammalian cells and *Leishmania* (Lux et al, 2002; Rakotomanga et al, 2007). The drug also inhibits cytochome oxidase activity in promastigotes. (Luque-Ortega and Rivas, 2007). In *L. donovani* promastigotes Miltefosin has been shown to significantly reduce the phosphatidylcholine content and to increase the phosphatidylethanolamine (PE) content in parasite membranes suggesting that the drug may cause a partial inactivation of PE-N-methyltransferase (Rakotomanga et al, 2007). Inhibition of N-methyltransferase activity by hexadecylphosphocholine has also been described in tumor cells (Jimenez-Lopez et al., 2003). This enzyme was only found in the *L. major* genome but interestingly it is absent in *T. cruzi* and *T. brucei* (see above). This may explain why Miltefosin only finds application as a drug in leishmaniasis and not in trypanosomiasis. In hepatocytes the Miltefosine molecule appeared to be slowly degraded by phospholipase-D-like cleavage resulting in the release of choline. Although a phospholipase D activity involved in signal transduction has been reported in the case of *L. donovani* (Blum et al., 2001; Rakotomanga et al, 2007), a corresponding gene was neither detected in the *L. major* genome, nor in that of *L. infantum* or *L. brasiliensi*s and the two trypanosomes *T. brucei* and *T. cruzi* (see table). This suggests that such an activity in *Leishmania* may be catalysed by a non-homologous enzyme. Also a phospholipase C gene was not detected.

If indeed *Leishmania* possesses such an isofunctional protein with phospholipase activity, but without the ability to degrade this lysophospholipid analogue, then this would be additional factor contributing to the efficacy of this drug against the parasite.

Amino-acid metabolism

(i) catabolism

Amino acids (particularly proline, threonine and glutamic acid may serve as important energy substrates for *Leishmania* in the midgut of the fly vector. L-Proline, the most abundant amino acid is oxidized to glutamate via mitochondrial delta-1-pyrroline-5-carboxylate dehydrogenase and pyrroline-5-carboxylate synthetase. The resulting glutamic acid is deaminated by a mitochondrial NAD-dependent glutamate dehydrogenase and then fed into the TCA cycle. All three trypanosomatids have a mitochondrial NAD-dependent glutamate dehydrogenase, but *T. cruzi* and *L. major* have an additional gene for a NADP-dependent isoenzyme which is probably present in the cytosol because it lacks a recognisable targeting peptide. This enzyme is closely related to the enzyme from gamma-proteobacteria and has probably entered via lateral gene tranfer. Aspartic acid is transaminated by a mitochondrial aspartate aminotransferase to oxaloacetate and fed into the cycle as well. Aspartate may also be converted to fumarate via a functional purine-nucleotide cycle, comprising the enzymes adenylosuccinate synthase, adenylosuccinate lyase and AMP-deaminase.

Glycine can be split by the glycine cleavage system into carbon dioxide and formic acid. Methionine is first converted in the cytosol to 2-ketobutyrate and the latter is converted in the mitochondrion to the TCA-cycle intermediate succinyl CoA. Isoleucine and valine, after transamination in the cytosol to their corresponding ketocarboxylic acids by a branched-chain aminotransferase, can be further oxidised in the mitochondrion via a short/branched-chain acyl-CoA dehydrogenase and a hydratase, to succinyl CoA and acetyl CoA. These enter the TCA cycle or the cycle catalysed by succinyl-CoA ligase and acetate: succinate CoA transferase, leading to the formation of acetate and ATP.

Branched-chain amino-acid metabolism has been the subject of several studies in *Leishmania* (Blum, 1991; Ginger et al., 1999; Ginger et al., 2000; Ginger et al., 2001). In animals and plants branched-chain amino-acid metabolism occurs by a mitochondrial pathway and in trypanosomatids metabolism of these ketogenic amino acids provides substrates for the TCA and ASCT cycles. Some genes required for branched-chain amino-acid metabolism (branched-chain 2-ketoamino-acid decarboxylase and 3-hydroxy-3-methylglutaryl (HMG) CoA lyase) encode potential mitochondrial transit peptides. In addition to a possible role as an energy-generating substrate, leucine, which is metabolised through the intermediacy of HMG-CoA, can also be incorporated directly into *Leishmania*'s sterol biosynthetic pathway (Ginger et al., 2000; Ginger et al., 2001). This direct route from leucine into the mevalonate pathway (which is the sole route used for isoprenoid biosynthesis in the trypanosomatids) has not been observed to operate in any other eukaryote, but is the major route for sterol biosynthesis for several *Leishmania* species, at least in cultured promastigotes. Previous immunolocalisation of trypanosomatid HMG-CoA reductase, the key enzyme required for mevalonate formation, indicated that this enzyme is present in the mitochondrion (Pena-Diaz et al., 2004). Thus this organelle seems to be a sub-cellular location for both HMGCoA

reductase and leucine catabolism, which may be the explanation for the incorporation of leucine carbon into sterol by trypanosomatids.

Most of the genes of the classical pathways for aromatic amino-acid oxidation are missing. Phenylalanine can be converted to tyrosine by a hydroxylase which is not present in the other trypanosomatids Some genes from the anaerobic phenylalanine degradation pathway are present. Tyrosine is most likely converted to 4-hydroxyphenylpyruvate by two enzymes, a true tyrosine aminotransferase, probably also acting on phenylalanine and tryptophan, and an aspartate aminotransferase which in *T. cruzi* has been shown to have a broad substrate specificity and is capable of acting on tryptophan as well (Vernal et al., 1998). The corresponding keto acids are most likely reduced by an aromatic hydroxyacid dehydrogenase, closely related to malate dehydrogenases. In *T. cruzi* this enzyme has been shown to have changed its substrate specificity and is called an aromatic alpha-hydroxyacid dehydrogenase (Vernal et al., 2002). A homologue is present in the *L. major* genome as well. No enzymes of the classical pathway converting tyrosine to fumarate and acetoacetate were detected.

Leishmania contains several enzymes of the urea cycle, although a fully functional urea cycle is missing from *L. major*. The possible functions of these enzyme is discussed in the section oxidant stress protection below.

Leishmania lacks the enzymes of the major route of threonine degradation in mammals which includes the enzymes threonine dehydrogenase and aminoacetone synthase. Instead it has two alternative pathways. In the first one, it can convert threonine through a tetrahydrofolate (THF)-dependent pathway to glycine using the enzyme serine hydroxymethyltransferase (SHMT) (also known as threonine aldolase). In addition to the demethylation of serine, SHMT is able to cleave the Calpha–Cbeta bond of threonine. The resulting glycine can be converted through serine to pyruvate by the THF-dependent glycine cleavage system (GCS) followed by a serine/threonine dehydratase (STD). Alternatively, the same dehydratase might convert threonine to 2-ketobutyrate, which is then oxidized to succinyl CoA. *T. brucei* lacks the dehydratase and SHMT, and seems to metabolize threonine using the alternative aminoacetone pathway, which involves a mitochondrial threonine dehydrogenase (TDH) and an aminoacetone synthase (2-amino-3-ketobutyrate CoA ligase). Thus despite the fact that here threonine metabolism differs from that in the host this would be less suitable as drug target because of the presence in *Leishmania* of alternative possibilities.

(ii) synthesis

The capacity for the synthesis of amino acids by *L. major* is limited to the non-essential amino acids *plus* threonine and methionine. The gene for D-3-phosphoglycerate dehydrogenase, the first enzyme committed to the synthesis of serine was found in *L. major* and a possible phosphoserine phosphatase homologue was detected with a BlastP e value of 3e-5. Cysteine can be produced either from homocysteine by the trans-sulfuration pathway or by *de novo* synthesis from serine. Glycine can be formed from serine by a serine hydroxymethyl transferase. Alanine is formed from pyruvate by a cytosolic alanine aminotransferase and aspartate and asparagine can be formed from oxaloacetate by a mitochondrial aspartate aminotransferase and an asparagine synthase.

Glutamate, glutamine and proline can be formed from 2-ketoglutarate. The genes for all the necessary enzymes were found. Of the so-called essential amino acids all genes coding for the enzymes for the synthesis of threonine were detected, but neither the aromatic amino acids (phenylalanine, tyrosine or tryptophan) nor any of the branched amino acids (leucine, isoleucine and valine) can be synthesised by *L. major*. Lysine and histidine cannot be formed as well. Methionine can both be synthesised *de novo* from homoserine or salvaged from homocysteine.

Purine salvage and pyrimidine synthesis

Several studies have shown that Trypanosomatidae are incapable of *de novo* purine synthesis, in common with other obligate parasitic protozoa (reviewed in: Marr and Berens, 1983). This has now been confirmed by the release of the trypanosomatid nuclear genome sequences. Only one of the 10 enzymes required to make inosine monophosphate (IMP) from phosphoribosyl pyrophosphate, adenylosuccinate lyase, is encoded in the *L. major* genome, and its presence is explained by the fact that this enzyme also plays a role in purine salvage by converting IMP to AMP (Van den Berghe et al., 1997). Instead, genes encoding several enzymes involved in the interconversion of purine bases and nucleosides are present. Moreover, several of these purine salvage enzymes carry a PTS, confirming earlier claims that some of them are glycosomal (Hammond et al., 1981). Trypanosomatids are able to synthesise pyrimidines *de novo* from glutamine, bicarbonate and aspartate by a pathway that involves six genes – carbamoylphosphate synthase, aspartate carbamoyltransferase, dihydroorotase, dihydroorotate dehydrogenase and orotidine-5'-phosphate decarboxylase/orotate phosphoribosyltransferase. In the *L. major* genome these genes are all present on chromosome 16 and tandemly linked. In mammals the first three enzyme activities are present as a multifunctional protein that arose as a consequence of gene fusions. The dihydroorotate product of these reactions is oxidised by a cytosolic dihydroorotate dehydrogenase, and the last two steps of pyrimidine synthesis are catalysed inside glycosomes by orotidine-5'-phosphate decarboxylase/orotate phosphoribosyltransferase, the genes for which are fused to encode a bifunctional protein. Interestingly, the orientation of enzyme activities on this fusion protein is opposite to the orientation present on the analogous multifunctional enzyme that is found in higher eukaryotes (Gao et al., 1999). Thus far, this “reverse fusion” is only found to be shared with the cyanobacteria, and may therefore be relevant to any discussion of the impact that lateral gene fusion has had upon the evolution of metabolism in the Trypanosomatidae (see below). In all eukaryotes, except the Kinetoplastida and yeasts, the enzyme dihydroorotae dehydrogenase is also intimately associated with the respiratory chain and utilises ubiquinone as electron acceptor for the oxidation of dihydroorotate to orotic acid. *Leishmania* and all other Kinetoplastida (including the Bodonidae) have lost this enzyme in exchange for a cytosolic isofunctional enzyme of bacterial origin, which utilizes fumarate as electron acceptor. It has recently been shown that erythrocytic stages of the human malaria parasite *Plasmodium falciparum* maintain an active mitochondrial electron transport chain to serve just the regeneration of ubiquinone required for the proper functioning of this enzyme. Expression of the soluble yeast dihydroorotate dehydrogenase rendered *Plasmodium* completely resistant to inhibitors of mitochondrial electron transport (Painter et al.,

2007). This observation is of utmost importance, not only because it illustrates the vital role of this enzyme for the survival of a parasite and its potential as a drug target, but also because it helps us to understand a peculiarity of the biology of the trypanosomatids; it explains why dyskinetoplastic trypanosomatids, which have lost large parts of their mitochondrial genome and lack a functional mitochondrial respiratory chain, do occur in nature. The *L. major* enzyme has been overexpressed and characterized (Feliciano et al., 2006) and the crystal structures of the corresponding *T. cruzi* (Inaoka et al., 2005) and *T. brucei* enzyme (2B4G) with bound orotic acid and flavin mononucleotide have been solved. RNA interference against expression of the dihydroorotate dehydrogenase gene in bloodstream forms of *T. brucei* resulted in inhibition of parasite growth in culture medium depleted of pyrimidines (Fred Bruckner, personal communication). Thus dihydroorotate dehydrogenase inhibitors can be useful, but only when combined with inhibitors of pyrimidine transport.

Protection against oxidant stress

(i) Enzymes of urea cycle and putrescine formation

In all trypanosomatids amino-acid catabolism leads to the formation of free ammonia which is excreted, but *Leishmania, Crithidia and Leptomonas* excrete urea, in addition to ammonia, which could be an indication for the presence of a functional urea cycle (Yoshida and Camargo, 1978). Indeed the *L. major* genome contains several genes for enzymes of the urea cycle which apparently have been acquired by horizontal gene transfer (*i.e.* an argininosuccinate synthase of clear bacterial signature and an arginase which clusters with 100% bootstrap support with the enzymes found in fungi). The latter enzyme is normally cytosolic, but in *Leishmania* it is present in the glycosomes (Roberts et al., 2004). A fully functional urea cycle seems to be missing (see Table 7 and Fig. 3). *L. major* lacks the genes coding for ornithine carbamoyltransferase and argininosuccinate lyase while *T. brucei and T. cruzi* lack all enzymes of the cycle. Thus in *Leishmania* arginine, ornithine and urea can be formed and this explains why *Leishmania* excretes urea when arginine is added to the growth medium, while arginine addition to members of the genus *Trypanosoma* does not lead to production of urea (Yoshida and Camargo, 1978). It has been suggested that the production of urea by *Leishmania* may be a special case of adaptation to life in an acidic phagolysosome because the release of ammonia in quantity could severely affect the vacuolar pH (Opperdoes and Coombs, 2007), but there may be another explanation for the acquisition of a few urea cycle enzymes by *Leishmania*.

Interferon-gamma, a Th1 cytokine normally associated with classical macrophage activation and killing of intracellular pathogens, induces a cationic amino-acid transporter, which is responsible for cytokine-stimulated import of **L**-arginine by the macrophage. Macrophages require L-arginine for the production of nitric oxide (NO) by another inducible enzyme: NO synthase. Activated macrophages also produce the superoxide radical which combines with NO to form the even more toxic peroxynitrite ($OONO^-$). The latter rapidly decomposes into the highly reactive hydroxyl radical, $OH^\cdot$ and NO_2. $OH^\cdot$ radicals effectively kill the ingested pathogens. However, *Leishmania* survive this killing attack and the addition of arginine to infected macrophages even leads to a stimulation of parasite growth (Wanasen et al., 2007). Little is known as to how

Leishmania accomplishes this. The *Leishmania* genome gives some insight into this and may even provide an explanation how macrophage stimulation may support the growth of the intracellular parasite. The *L. major* genome has revealed the presence of a high affinity arginine transporter with acidic pH optimum which is most likely expressed in the intracellular stage (Shaked-Mishan et al., 2006). This permease would allow the amastigote to scavenge from the macrophage the arginine destined for NO production and then, via its glycosomal arginase, neutralize the arginine by converting it into ornithine and urea. Ornithine is normally broken down via glutamate to ketoglutarate, but there is no evidence that this pathway operates in any of the trypanosomatids. One of the necessary enzymes, ornithine aminotransferase, was not detected. Yet in view of the stimulation of parasite growth by arginine it is tempting to assume that ornithine can be metabolized by an as yet unknown pathway. If not, ornithine is most likely excreted, or converted to polyamines, which either are utilized for trypanothione synthesis (see below) or excreted. Thus it is hypothesized that the function of the arginine transporter together with the glycosomal arginase are the lowering of the phagosomal arginine concentration by which the amastigote is able to protect itself against production of NO inside the phagosomes.

NO is a diatomic gaseous molecule similar to dioxygen and carbon monoxyde. These molecules can all be bound by haemoproteins such as haemoglobin and cytochromes. Indeed, in mammalian cells NO is known to function as a signal transducer by binding to a haem-containing high-affinity-binding site of a soluble guanylate cyclase (sGC) and so regulates cGMP formation. NO forms a tight 5-coordinate haem adduct with sGC (Lawson et al., 2000). Thus haem's strong affinity for NO may explain why *Leishmania*, in addition to two enzymes of the urea cycle, also has acquired by lateral gene transfer, several genes involved in haem biosynthesis. Maybe, in addition to the reduction of phagosomal arginine concentrations by the parasite's arginase, an as yet unidentified haemoprotein functions as an effective sink for any NO still being produced by the macrophage.

Thus if confirmed, arginase and the hypothetical haemoprotein could be interesting drug targets. Creation of arginase-deficient mutant parasites has indeed established that the enzyme is essential for *L. mexicana* promastigotes, but this is because they become auxotroph for ornithine (Roberts et al., 2004). Unfortunately the effect of such deletion on the survival of amastigotes inside macrophages was not tested.

(ii) Polyamines

Putrescine can be synthesized by decarboxylation of ornithine (see above and section on oxidative stress)), both in *Leishmania* and in *T. brucei*. However, an ornithine decarboxylase is absent from *T. cruzi.* There is some controversy as to whether in *T. cruzi* putrescine is synthesised by an alternative pathway comprising arginine decarboxylase plus agmatinase, instead of by the obviously absent ornithine decarboxylase (Hernandez et al.,1999). However, while a gene coding for an agmatinase is present an arginine decarboxylase was not found in any of the trypanosomatids. This is in good agreement with the notion that *T. cruzi* is auxotrophic for polyamine and is able to salvage spermidine from the medium (Carillo et al., 2003).

Polyamines are essential for cell growth and differentiation. The amino acids arginine and ornithine are precursors for polyamine biosynthesis, and are necessary for the synthesis of trypanothione (Schmidt & Krauth-Siegel, 2002), a dithiol unique to trypanosomatids and which has a major role in protection against oxidative stress. The polyamine biosynthetic pathway is a potential target for drugs against various diseases caused by these protozoan parasites. The target has been validated using different enzyme inhibitors (Müller et al., 2001), as well as null mutants of *L. donovani* (Roberts et al., 2002, Jiang et al., 1999, Roberts et al., 2001). The first step in polyamine biosynthesis is carried out by ornithine decarboxylase and this enzyme is the target for the drug difluoromethylornithine (DFMO, eflornithine) in human sleeping sickness (Phillips et al., 1987). The enzyme is also present in *L. major* (see also the section on the urea cycle). However, DFMO which irreversibly inactivates its target enzyme, is not very effective against trypanosomatids other than *T. brucei,* indicating important differences. Indeed the *T. brucei* enzyme is the result of a relatively recent event of horizontal gene transfer from a vertebrate to an African trypanosome and is distantly related to the enzyme from the other trypanosomatids (Steglich and Schaeffer, 2005). Another difference may be in the half life of ornithine decarboxylase. A difference in turnover of ornithine decarboxylases from various trypanosomatids has been demonstrated and the *C. fasciculata* enzyme seems especialy sensitive to degradation. Whether this is also the case for the *leishmania* enzyme is likely but remains to be demonstrated (Persson, 2007). S-Adenosine methionine decarboxylase catalyses the production of decarboxylated adenosyl methionine, which provides the aminopropyl groups in the synthesis of spermidine and spermine from putrescine and spermidine respectively. S-Adenosine methionine decarboxylase in trypanosomatids has been demonstrated to be a potential target for drugs, but the information about the enzyme is still limited. A candidate aminopropyltransferase and a trypanothione synthetase are present in *L. major* as well.

Cysteine can be produced from homocysteine by the trans-sulfuration pathway present in all three organisms. *De novo* synthesis from serine appears possible in *L. major*. The enzyme responsible for the conversion, cysteine synthase, is a typical plant-like enzyme, further only found in cyanobacteria, algae and plants. *L. major* can also interconvert glycine and serine via serine hydroxymethyl transferase. Methionine (as decarboxylated AdoMet), with cysteine, glutamate and glycine are the precursor amino acids for glutathione. Although the latter is present in trypanosomatids, it is also used, together with spermidine, for the synthesis of trypanothione. This unique thiol has taken over many of the protective, metabolic and antioxidant functions of glutathione.

(iii) Trypanothione

Trypanothione is essential for the defence against chemical and oxidant stress in *Leishmania*. It is also the target for trivalent antimonial drugs which form conjugates with trypanothione and/or glutathione (Fairlamb, 2003). Methylglyoxal, a reactive by-product of the glycolytic pathway, can be converted via a trypanothione-dependent glyoxalase pathway (Vickers et al., 2004) to D-lactate, an end product of leishmania carbohydrate catabolism. Homologues of the *T. cruzi* glyoxalase I and II are present in *L. major*. See

the section on the methyl glyoxal bypass above.

(iv) Superoxide dismutase

Leishmania has four superoxide dismutases (SODA, two SODBs and an SOD-C). They are all of the iron type (Dufernez et al., 2005). These Fe-SODs are typical prokaryotic enzymes and again could have entered the Trypanosomatidae by one or more events of horizontal transfer followed by gene duplications (Dufernez et al., 2005). SODA and C are putative mitochondrial isoenzymes and the SODBs are predicted to be glycosomal and cytoplasmic, respectively. There is no evidence for the presence of either a Mn-dependent enzyme (Dufernez et al., 2005) or Cu/Zn-superoxide dismutase. Several SOD deletion mutants have been generated. In general such mutants have shown to be viable, although they exhibit an increased sensitivity to oxidative stress. All pathogenic trypanosomatids lack catalase. For removal of peroxides they are dependent on trypanothione-linked peroxidases. Tryparedoxins are homologous to thioredoxin and trypanosomes contain multiple copies of both kinds of redox protein. Thioredoxin reductase is absent and the trypanosomatid peroxidases are coupled to trypanothione/trypanothione reductase (Opperdoes and Szikora, 2006).

(v) NADPH production

Promastigotes of glucose-transporter null-mutants have a reduced capacity to generate the NADPH required for protection against reactive oxygen species and are significantly more susceptible to oxidative stress. (Rodriguez-Contreras et al., 2007), suggesting that enzymes of the oxidative branch of the pentose-phosphate pathway play a vital role in these cells. It is likely that this is even more the case for amastigotes which, in the phagosome, are exposed even much higher levels of oxidative stress (Haas, 2007). However, because of the fact that in *Leishmania* multiple copies of the various enzymes involved in oxidative stress protection were identified this may not be an easy drug target (see above).

(iv) Ascorbate metabolism

Almost all animals synthesize ascorbate but primates (including humans) need vitamin C (ascorbic acid) as an external source. Ascorbate is essential for the production and maintenance of collagen, the hydroxylation of amino acids and the defense against oxidants. Trypanosomatids have no collagen. Since the only amino acid hydroxylation reaction identified in *Leishmania* is the conversion of phenylalanine into tyrosine, a reaction that does not occur in the trypanosomes and whose function in *L. major* is far from clear, ascorbate's only obvious function in trypanosomatids should be oxidative stress protection. This is supported by the presence of an ascorbate-dependent peroxidase in *L. major* and *T. cruzi* (Wilkinson et al., 2002). *Leishmania* amastigotes survive inside the phagosome of the macrophage, which is a hostile environment known to be the site of generation of reactive oxygen species. Genome mining has identified a gulono-1,4-lactone oxidase homologue, the last enzyme in the formation of ascorbate. This enzyme has a PTS in both trypanosomes and *Leishmania*, strongly suggesting that it is a glycosomal enzyme. Experimental evidence obtained for trypanosomes indicates that a fungal type of ascorbate biosynthesis may be operational in their glycosomes (Wilkinson et al., 2005). Although many enzymes of both the animal-type and plant-type ascorbate

synthetic pathways leading to the synthesis of ascorbic acid could be identified, the database lacked one or more essential enzymes of each pathway. Moreover, the trypanosome enzyme has been shown to prefer **D**-arabino-1,4-lactone rather than gulono-1,4-lactone as substrate (Wilkinson et al., 2005). This is suggestive evidence for the operation of a yeast-type rather than an animal- or plant-type pathway in trypanosomatids, because the yeast pathway results in the formation of erythroascorbate from **D**-arabinose. Indeed, the trypanosomatid genomes contain a gene that encodes a homologue of NADP-dependent arabinose dehydrogenase which catalyses the formation of **D**-arabinono-1,4-lactone from **D**-arabinose (this enzyme is annotated as prostaglandin f2-alpha synthase, LmjF31.2150). The *Leishmania* enzyme also has a potential PTS, suggesting that erythroascorbate, rather than ascorbate, is generated within glycosomes as an antioxidant. **D**-arabinose is a rare sugar which is only known in polysaccharides of plants and so far there are no obvious candidate enzymes in trypanosomatids that are able to form **D**-arabinose from other C5 sugars. Interestingly, however, *L. major* has recently been reported to incorporate D-arabinose into lipophosphoglycan side chains (Dobson et al., 2003). Since humans (and primates) have lost the capacity to synthesize ascorbic acid, due to a mutation in the gulonolactone oxidase gene, the trypanosomatid enzyme may be an interesting drug target. It also remains to be seen whether the only known amino-acid hydroxylation reaction to occur in *L. major*, the conversion of phenylalanine to tryosine by phenylalanine, requires (erythro)ascorbate for catalysis. A thiol-dependent enzyme possibly involved in the reduction of dehydroascorbic acid has also been reported (Denton et al. 2004), Interestingly, it is up-regulated in amastigotes and could be involved in mediating sensitivity of this stage to antimonial drugs.

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Figure 1. Gluconeogenesis and related pathways in *Leishmania*. Boxed metabolites are nutrients (in gray) or end-products (in black) of metabolism. PPP, pentose-phosphate pathway. Enzymes: 1, hexokinase; 2, phosphoglucose isomerase; 3a, phosphofructokinase; 3b, fructose-1,6-bisphosphatase ; 4, fructosebisphosphate aldolase; 5, triosephosphate isomerase; 6, glyceraldehyde-3-phosphate dehydrogenase; 7, phosphoglycerate kinase; 8, glycerol-3-phosphate dehydrogenase; 9 glycerol kinase; 10, adenylate kinase; 11, glucosamine-6-phosphate deaminase; 12, mannose-6-phosphate isomerase; 13, phosphomannomutase; 14, GDP-mannose pyrophosphorylase; 15, phosphoglycerate mutase; 16, enolase; 17, pyruvate kinase; 18, phosphoenolpyruvate carboxykinase; 19, malate dehydrogenase; 20, fumarate hydratase; 21, NADH-dependent fumarate reductase; 22, pyruvate phosphate dikinase; 23, alanine aminotransferase; 24, amylase-like protein; 25, sucrase-like protein.

Figure 2. Glyoxalase pathway. Enzyme names are in italics. Glyoxalase I is absent from *T. brucei*. D-lactate dehydrogenase is only present in *Leishmania*

Figure 3. Enzymes of the urea cycle and polyamine biosynthesis in *Leishmania*. Broken arrows represent enzymes for which the corresponding genes were not detected. Enzyme names are in italics

Table 1. Enzymes of carbohydrate metabolism of *L.major*

Enzyme name	Pathway	GeneDB accession
2,3-bisphosphoglycerate-independent phosphoglycerate mutase	glycolysis gluconeogenesis	LmjF36.6650
2,3-bisphosphoglycerate-independent phosphoglycerate mutase	glycolysis gluconeogenesis	LmjF28.2220
2-oxoglutarate dehydrogenase e1 component, mitochondrial precursor, putative	krebs	LmjF36.3470
2-oxoglutarate dehydrogenase, e3 component, lipoamidedehydrogenase-like	krebs	LmjF31.2640
2-oxoglutarate dehydrogenase, e3 component, lipoamidedehydrogenase-like	krebs carbohydrate	LmjF31.2640
aconitase, probable	krebs	LmjF18.0510
ACPM, acyl carrier protein, putative	fatty acid synthesis | NADH deydrogenase | respiratory chain	LmjF27.0290
adenylate kinase	glycolysis	LmjF36.1360
adenylate kinase, putative|Leishmania major	glycolysis	LmjF21.1250
Alanine amino transferase putative, hypothetical 55.0 Kd protein, probable	glycolysis amino-acid metabolism pyruvate alanine	LmjF12.0630
aquaglyceroporin	glycolysis glycerol fatty acid	LmjF31.0020
ATPase alpha subunit	ATPase|respiratory chain|complex V | mitochondrial	LmjF05.0510
ATPase beta subunit, putative	ATPase|respiratory chain|complex V| mitochondrial	LmjF25.1180
ATPase epsilon subunit, possible	ATPase|respiratory chain|complex V| mitochondrial	LmjF36.0620
beta-fructosidase-like	glycolysis carbohydrate	LmjF23.0870
citrate lyase beta chain / citryl-coa lyase subunit-like	krebs |fatty acid synthesis	LmjF24.1440
citrate synthase	krebs | fatty acid synthesis	LmjF18.0680
citrate synthase	krebs	LmjF18.0680
COX11|Cytochrome c oxidase assembly protein, putative	respiratory chain| mitochondrial	LmjF3.0100
cytochrome c oxidase subunit 5, probable	respiratory chain| mitochondrial	LmjF26.1710
cytochrome c oxidase subunit iv	respiratory chain| mitochondrial	LmjF12.0670
cytochrome c oxidase subunit vi	respiratory chain| mitochondrial	LmjF21.1710
cytochrome c, probable	respiratory chain| mitochondrial	LmjF16.1320
cytochrome c1 precursor-like	respiratory chain complex III| mitochondrial	LmjF36.3090
cytochrome c1 precursor-like	respiratory chain complex III| mitochondrial	LmjF13.0850
cytochrome c1, putative	respiratory chain complex III| mitochondrial	LmjF07.0060
electron transfer flavoprotein-ubiquinone oxidoreductase, putative	respiratory chain| mitochondrial	LmjF07.0600
enolase	glycolysis gluconeogenesis	LmjF14.1160
fructose-1,6-bisphosphate aldolase	carbohydrate glycolysis calvin gluconeogenesis	LmjF36.1260
Fumarate hydratase class I, anaerobic	krebs carbohydrate	LmjF29.1960
fumarate hydratase (glycosomal)	krebs carbohydrate	LmjF24.0320

galactokinase	carbohydrate galactose glycolysis	LmjF35.2740
glucokinase	carbohydrate |glycolysis	LmjF36.2320
glucosamine-6-phosphate deaminase	glycolysis	LmjF32.3260
glucose transporter, putative, probably LmGT1	glycolysis	LmjF36.6300
glucose-6-phosphate isomerase	carbohydrate glycolysis gluconeogenesis	LmjF12.0530
glyceraldehyde-3-phosphate dehydrogenase	carbohydrate glycolysis calvin gluconeogenesis	LmjF30.2970
glyceraldehyde-3-phosphate dehydrogenase	carbohydrate glycolysis calvin gluconeogenesis	LmjF30.2970
glycerol kinase	carbohydrate glycolysis	LmjF35.3080
glycerol-3-phosphate dehydrogenase, FAD dependent mitochondrial	carbohydrate glycolysis respiratory chain| mitochondrial	LmjF28.0240
glycerol-3-phosphate dehydrogenase (NAD)	carbohydrate glycolysis	LmjF10.0510
glycerol-3-phosphate dehydrogenase, possible mitochondrial	carbohydrate glycolysis respiratory chain| mitochondrial	LmjF20.0430
Hexokinase	glycolysis carbohydrate	LmjF21.0240
isocitrate dehydrogenase	krebs	LmjF33.2550
isocitrate dehydrogenase [NADP], mitochondrial precursor, putative	krebs	LmjF10.0290
isocitrate dehydrogenase [NADP], mitochondrial precursor, putative	Krebs cycle	LmjF10.0290
malate dehydrogenase mitochondrial MDH	mitochondrial | krebs | tca cycle	LmjF34.0140
Malate synthase	krebs carbohydrate glyoxylate	Absent
mannose-1-phosphate guanyltransferase| GDP-D-mannose pyrophosphorylase	glycosylation | ascorbate biosynthesis plants 4	LmjF23.0110
monocarboxylate transporter-like	glycolysis krebs	LmjF05.0480
NADH-dependent fumarate reductase, FRDg	krebs carbohydrate	LmjF35.1180
NADH-dependent fumarate reductase, putative	krebs carbohydrate	LmjF35.1180
NADH-ubiquinone oxidoreductase 20 Kd subunit, mitochondrial precursor, putative	respiratory chain| mitochondrial	LmjF27.0740
NDI1, NADH dehydrogenase, putative (rotenone insensitive subunit)	respiratory chain nadh| mitochondrial	LmjF36.5380
NI8M, hypothetical protein, conserved	NADH dehydrogenase | respiratory chain	LmjF32.3170
NUAM, NADH-ubiquinone oxidoreductase 75 Kd subunit, mitochondrial precursor(ec 1.6.5.3), probable	respiratory chain nadh| mitochondrial	LmjF18.1480
NUBM, NADH dehydrogenase	respiratory chain nadh| mitochondrial	LmjF05.0980
NUBM, NADH dehydrogenase, putative	respiratory chain nadh| mitochondrial	LmjF05.0980
NUEM, NADH dehydrogenase, possible	respiratory chain nadh| mitochondrial	LmjF18.0080
NUHM, NADH-ubiquinone oxidoreductase 24 kDa subunit, mitochondrial precursor-like protein	respiratory chain nadh| mitochondrial	LmjF17.0270
NUKM, NADH-ubiquinone oxidoreductase 20 Kd subunit, mitochondrial precursor, putative	respiratory chain nadh| mitochondrial	LmjF27.0740
phosphofructokinase	carbohydrate glycolysis	LmjF29.2510
phosphoglucomutase	ascorbate biosynthesis animals 1 | carbohydrate metabolism, glycosylation | glycolysis	LmjF21.0640
Phosphoglucomutase	carbohydrate metabolism gluconeogenesis glycosylation	LmjF21.0640

phosphoglycerate kinase, cytosolic	carbohydrate glycolysis calvin gluconeogenesis	LmjF20.0110
phosphoglycerate kinase, glycosomal	carbohydrate glycolysis gluconeogenesis	LmjF30.3380
phosphoglycerate kinase, glycosomal	carbohydrate glycolysis calvin gluconeogenesis	LmjF20.0100
phosphoglycerate kinase, glycosomal PGKC	carbohydrate glycolysis calvin gluconeogenesis	LmjF20.0100
phosphoglycerate mutase cofactor dependent-like	carbohydrate | glycolysis | gluconeogenesis	LmjF08.0060
phosphomannomutase	carbohydrate metabolism, glycosylation | ascorbate biosynthesis plants 3	LmjF34.3780
phosphomannomutase, putative	carbohydrate metabolism, glycosylation | ascorbate biosynthesis plants 3	LmjF36.1960
phosphomannose isomerase	glycolysis | carbohydrate | glycolipid ancer | ascorbate biosynthesis plants 2	LmjF32.1580
putative cytochrome c oxidase subunit vi	respiratory chain| mitochondrial	LmjF21.1710
putative ubiquinol--cytochrome-c reductase, probable	respiratory chain complex III | mitochondrial	LmjF31.2580
pyruvate carrier, mitochondrial possible	glycolysis krebs carbohydrate mitochondrion metabolite carrier	LmjF32.1110
pyruvate kinase	glycolysis	LmjF35.0020
reiske iron-sulfur protein precursor	respiratory chain mitochondrial| mitochondrial	LmjF35.1510
similarity with triose p/pi translocator cptr_soltu, possible	glycolysis calvin	LmjF22.1010
succinate dehydrogenase	krebs| mitochondrion	LmjF24.1630
succinyl-coa ligase alpha-chain, mitochondrial precursor), putative	krebs| mitochondrion	LmjF25.2140
succinyl-coa ligase beta subunit, putative	krebs | mitochondrion	LmjF36.2950
sucrase-like protein	glycolysis	LmjF27.2340
sugar transporter-like	glycolysis	LmjF24.0680
triosephosphate isomerase	glycolysis calvin gluconeogenesis	LmjF24.0850
ubiquinol-cytochrome c reductase complex core protein i-like protein	respiratory chain complex III| mitochondrial	LmjF35.1370
UTP-glucose-1-phosphate uridylyltransferase 2, putative	ascorbate biosynthesis animals 1 | glycosylation |	LmjF18.0990

Table 2. Trypanosomatid enzymes of the methylglyoxal bypass

Enzyme_name	**GeneDB accession**		
	T. brucei	***T. cruzi***	***L. major***
methylglyoxal synthase	Absent	Absent	Absent
GLO1‖glyoxalase I, trypanothione-dependent	none	Tc00.1047053510743.70	LmjF35.3010
glyoxalase II, trypanothione-dependent	Tb06.3A7.500	Tc00.1047053507603.230	LmjF12.0220
D-lactate dehydrogenase-like protein	Absent	Absent	LmjF27.2020

Table 3. Trypanosomatid peroxins identified in the TriTryp database

Peroxin	Function	Protein family	GeneDB Accession		
			T. bucei	T. cruzi	L. major
PEX1	import glycosomal matrix proteins	AAA+ family ATPase-like protein	Tb06.4F7.680	Tc00.1047053511745.20	LmjF30.2540
PEX2	import glycosomal matrix proteins	glycosomal membrane protein|RING-finger protein	Tb03.48O8.580	Tc00.1047053508479.230	LmjF25.2230
PEX4	import glycosomal matrix proteins	ubiquitin-conjugating protein family protein (E2)	Tb08.12O16.620	Tc00.1047053509607.10	LmjF07.0850
PEX5	import glycosomal matrix proteins	peroxisomal targeting signal-1 receptor|TPR protein	Tb05.28F8.110	Tc00.1047053508299.70	LmjF35.1420
PEX6	import glycosomal matrix proteins	AAA+ family ATPase-like protein	Tb05.6E7.930	Tc00.1047053506947.40	LmjF16.0060
PEX7	import glycosomal matrix proteins	peroxisomal targeting signal type 2 receptor|WD-repeat protein	Tb03.28C22.1010	Tc00.1047053506989.160	LmjF29.0740
PEX10	import glycosomal matrix proteins	glycosomal membrane protein|RING-finger protein	Tb03.48O8.510	Tc00.1047053508479.190	LmjF25.2290
PEX11	glycosome proliferation	glycosomal membrane protein	Tb11.01.3370	Tc00.1047053511017.50	LmjF28.2260
PEX12	import glycosomal matrix proteins	glycosomal membrane protein|RING-finger protein	Tb10.61.0440	Tc00.1047053503809.20	LmjF19.1250
PEX14	import glycosomal matrix proteins	glycosomal membrane protein	Tb10.100.0130	Tc00.1047053504069.50	LmjF21.1840
PEX19	insertion of glycosomal membrane proteins		Tb09.211.3300	Tc00.1047053510737.100	LmjF35.3260

Table 4. Enzymes involved in the synthesis of biotin, folate, FAD, NAD, thiamine pyrophosphate and pyridoxal phosphate

Pathways	Enzyme_name	EC_Number	Accession
biotin	biotin/lipoate protein ligase-like protein		LmjF31.1070
Coenzyme A biosynthesis 1	pantothenate kinase subunit, putative	2.7.1.33	LmjF28.0140
Coenzyme A biosynthesis 2	hypothetical protein, conserved|Phosphopantothenate--cysteine ligase (EC 6.3.2.5) (Phosphopantothenoylcysteine synthetase) (PPC synthetase)	6.3.2.5	LmjF25.1900
Coenzyme A biosynthesis 3	hypothetical protein, conserved|Phosphopantothenoylcysteine decarboxylase (EC 4.1.1.36) (PPC-DC) (CoaC)	4.1.1.36	LmjF30.1540
Coenzyme A biosynthesis 4	Phosphopantetheine adenylyltransferase|hypothetical protein, conserved	2.7.7.3	LmjF32.2070
Coenzyme A biosynthesis 5	dephospho-CoA kinase, putative	2.7.1.24	LmjF22.1530
flavine metabolism 1| FAD synthesis|cofactor| vitamine b2	riboflavin kinase/fmn adenylyltransferase-like protein	2.7.1.26	LmjF35.3160
flavine metabolism 2| FAD synthesis|cofactor | vitamine b2	phosphoadenosine phosphosulfate reductase-like protein| FAD synthetase (EC 2.7.7.2) (FMN adenylyltransferase) (FAD pyrophosphorylase) (Flavin adenine dinucleotide synthetase)	2.7.7.2	LmjF28.0270
folate metabolism |amino acid threonine serine glycine pyruvate |cofactor	glycine dehydrogenase	2.1.2.10	LmjF26.0030
folate metabolism |amino acid threonine serine glycine pyruvate |cofactor	serine hydroxymethyltransferase, cytosolic	2.1.2.1	LmjF14.1320
folate metabolism | amino acid threonine serine glycine pyruvate|cofactor	serine hydroxymethyltransferase, mitochondrial	2.1.2.1	LmjF28.2370
folate metabolism | biopterin |pteridin|pterin|amino acid||cofactor	QDPR||quinonoid dihydropteridine reductase (7 copies)	1.5.1.34	LmjF34.4510
folate metabolism	biopterin transporter, putative (12 copies)		LmjF35.5150
folate metabolism	DHFR-TS||dihydrofolate reductase-thymidylate synthase	1.5.1.3|2.1.1.45	LmjF06.0860
folate metabolism	FTHS (c-1-tetrahydrofolate synthase, cytoplasmic, putative)	6.3.4.3|	LmjF30.2600
folate metabolism	methionine synthase, putative	2.1.1.13	LmjF07.0090
folate metabolism	methionyl-trna formyltransferase, putative	2.1.2.9	LmjF32.2240
Folate metabolism	methylenetetrahydrofolate dehydrogenase-like protein	1.5.1.5	LmjF22.0340
folate metabolism	methylenetetrahydrofolate reductase, putative	1.5.1.20	LmjF36.6390
folate metabolism	MTFC/MTFD (C-1-tetrahydrofolate synthase, cytoplasmic, putative)	3.5.4.9|1.5.1.5	LmjF26.0320
folate metabolism	pteridine transporter ft5, putative		LmjF35.5150
folate metabolism	pteridine reductase PTR1	1.5.1.33	LmjF23.0270
folate metabolism	FPGS||folylpolyglutamate synthetase	6.3.2.17	LmjF36.2610
folate metabolism| glycine cleavage system|cofactor	aminomethyltransferase, mitochondrial precursor, putative (subunit of the glycine cleavage system) (2 copies)	2.1.2.10	LmjF36.3810
folate metabolism glycine	glycine cleavage system H protein, putative	2.1.2.10	LmjF35.4720

formate|cofactor			
folate metabolism| Pteridine| |biopterin	pterin-4-alpha-carbinolamine dehydratase, putative	4.2.1.96	LmjF11.0220
NAD biosynthesis 1	nicotinate phosphoribosyltransferase, putative	2.4.2.11	LmjF33.0960
NAD biosynthesis 2|cofactor	Nicotinamide mononucleotide adenylyltransferase 2 (EC 2.7.7.1)| NAD(+) pyrophosphorylase	2.7.7.1	LmjF17.1340
NAD biosynthesis 3	NAD+ synthase	6.3.1.5	LmjF33.0960
NAD biosynthesis 4| NADP	ATP-NAD kinase-like protein	2.7.1.23	LmjF06.0460
NAD biosynthesis | tryptophan	kynureninase, possible	3.7.1.3	LmjF26.2240
pyridoxal phosphate synthesis | vitamine b6	pyridoxal kinase, putative	2.7.1.35	LmjF30.1250

Table 5. Enzymes of lipid metabolism in trypanosomatids

Pathways	**Enzyme name**	**EC Number**	**GeneDB accession**		
			T. brucei	***T. cruzi***	***L. major***
ether lipid	alkyldihydroxyacetonephosphate synthase (ec 2.5.1.26) (alkyl-dhapsynthase) (alkylglycerone-phosphate synthase)		Tb06.28P18.170	Tb06.28P18.170	LmjF30.0120
fatty acid lipid	3,2-trans-enoyl-coa isomerase		228.m01202 |Tb08.30P3. temporary.140|RH73277p	7212.m00004 |Tc00.1047053507107.40	LmjF31.2330
fatty acid | lipid metabolism beta oxidation	acyl-coa dehydrogenase (medium chain specific)	1.3.99.3	291.m00026 |Tb11.55.0026 ?	8359.m00029 |Tc00.1047053510303.290	LmjF06.0880
fatty acid synthesis lipid	beta-ketoacyl synthase family protein	2.3.1.41	199.m00383 |Tb927.2.3910|28H13.305	4937.m00002 |Tc00.1047053504157.20	LmjF33.2720
lipid	2,4-dienoyl-coa reductase-like	4.2.1.17 | 1.1.1.35	227.m00864 |Tb07.10C21.490	8359.m00021 |Tc00.1047053510303.210	LmjF06.0930
lipid	3,2-trans-enoyl-coa isomerase, mitochondrial precursor-like	4.2.1.17 | 1.1.1.35	169.m00967 |Tb04.3M17.150	7212.m00004 |Tc00.1047053507107.40	LmjF31.2330
lipid	3-hydroxyacyl-coa dehydrogenase family-like protein (trifunctional enzyme)	4.2.1.17 | 1.1.1.35	Tb927.2.4130	7378.m00004	LmjF33.2600
lipid	enoyl-CoA hydratase/Enoyl-CoA isomerase/3-hydroxyacyl-CoA dehydrogenase, putative bifunctional or trifunctional enzyme	4.2.1.17 | 1.1.1.35	199.m00405 |Tb927.2.4130|28H13.415	7730.m00007 |Tc00.1047053508441.70	LmjF33.2600
lipid	peroxisomal enoyl-CoA hydratase	4.2.1.17	224.m00842 |Tb03.48K5.550	8643.m00021 |Tc00.1047053511277.210	LmjF18.0580
lipid	peroxisomal enoyl-CoA hydratase	4.2.1.17	224.m00842 |Tb03.48K5.550	8643.m00021 |Tc00.1047053511277.210	LmjF18.0580
lipid	peroxisomal enoyl-CoA hydratase	4.2.1.17	none	8643.m00021 |Tc00.1047053511277.210	LmjF18.0580
lipid	peroxisomal enoyl-coa hydratase, probable	4.2.1.17 | 1.1.1.35 | 5.3.3.-)	none	8643.m00021 |Tc00.1047053511277.210	LmjF18.0580
lipid	possible mitochondrial trifunctional enzyme alpha subunit precursor		199.m00405 |Tb927.2.4130|28H13.415	7378.m00004 |Tc00.1047053507547.40	LmjF33.2600
lipid isoprenoids sterol	mevalonate kinase	2.7.1.36	Tb04.1D20.470	8046.m00001	LmjF31.0560
Lipid metabolism	3-ketoacyl-coa thiolase, possible	2.3.1.16	228.m00365 |Tb08.26A17.500	8107.m00003 |Tc00.1047053509463.30	LmjF23.0690
Lipid metabolism	3-ketoacyl-coa thiolase, possible		228.m00365 |Tb08.26A17.500	8107.m00003	LmjF23.0690

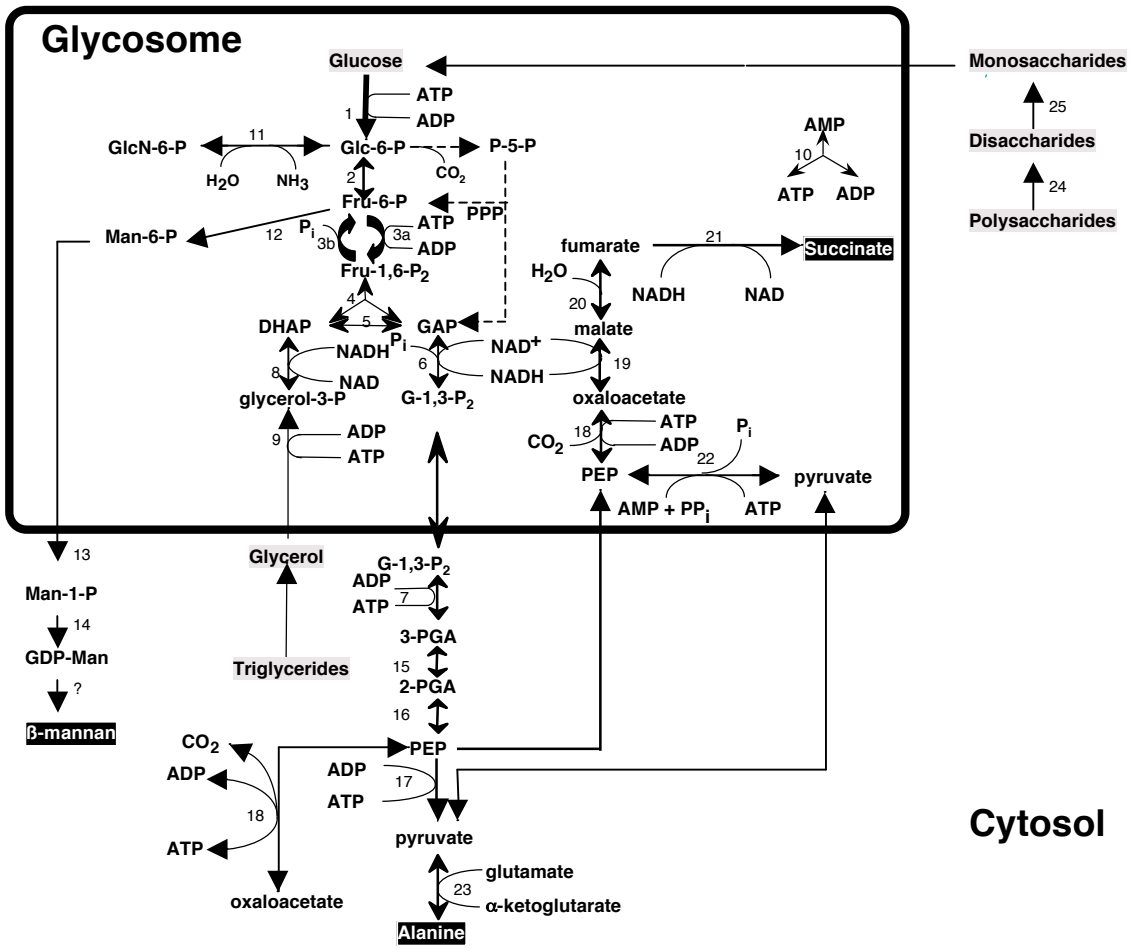
				||Tc00.1047053509463.30	
Lipid metabolism	acetyl-coenzyme a carboxylase like carboxylase, possible		228.m01122 ||Tb08.10K10.720	7702.m00005 ||Tc00.1047053508369.50	LmjF31.2970
lipid metabolism	acyl-coa binding protein, probable		Tb04.29M18.660	Tc00.1047053510877.55	LmjF34.2600
lipid metabolism	acyl-coa dehydrogenase, putative (very long chain)	1.3.99.3	237.m00364 ||Tb11.01.3640	7887.m00004 ||Tc00.1047053508827.40	LmjF28.2510
lipid metabolism	acyltransferase		Tb03.48K5.450	Tc00.1047053508153.100	LmjF29.2280
lipid metabolism	Beta-ketoacyl (ACP) synthase family protein	2.3.1.41	199.m00383 |Tb927.2.3910	8600.m00012 ||Tc00.1047053511109.120	LmjF33.2720
lipid metabolism	beta-ketoacyl synthase family protein, putative, probable	2.3.1.42	199.m00383 |Tb927.2.3910|28H13.305	8600.m00012 ||Tc00.1047053511109.120	LmjF33.2720
Lipid metabolism	c-8 sterol isomerase		Tb03.48K5.130	8364.m00009	LmjF29.2140
Lipid metabolism	c-8 sterol isomerase		224.m00800 ||Tb03.48K5.130	8364.m00009 ||Tc00.1047053510329.90	LmjF29.2140
Lipid metabolism	fatty acid elongase (very long chain)		131.t00014	Tc00.1047053509539.30	Tb05.26C7.100
Lipid metabolism	thiolase protein-like (most likely trifunctional protein Beta subunit)		none	8680.m00015 ||Tc00.1047053511389.150	LmjF31.1640
lipid metabolism beta oxidation	acyl-coa dehydrogenase (long-chain specific)	1.3.99.3	291.m00026 ||Tb11.55.0026	7029.m00022 ||Tc00.1047053506629.220	LmjF27.0930
lipid metabolism beta oxidation	acyl-coa dehydrogenase (short/branched chain specific)	1.3.99.3	291.m00026 ||Tb11.55.0026 ?	7168.m00008 ||Tc00.1047053507001.80	LmjF35.2730
lipid metabolism ether-lipid	alkyl dihydroxyacetonephosphate synthase	2.5.1.26	Tb06.28P18.170	4766.m00001	LmjF30.0120
lipid metabolism ether-lipid	alkyldihydroxyacetonephosphate synthase	2.5.1.26	226.m00335 ||Tb06.28P18.170	6711.m00011 ||Tc00.1047053505807.110	LmjF30.0120
lipid metabolism ether-lipid	DHAP acyltransferase	2.3.1.42	169.m00598 ||Tb04.2H8.550	4886.m00004 ||Tc00.1047053504055.40	LmjF34.1090
lipid metabolism ether-lipid	dihydroxyacetone phosphate acyltransferase, possible	2.3.1.42	169.m00598 ||Tb04.2H8.550	4886.m00004 ||Tc00.1047053504055.40	LmjF34.1090
lipid metabolism ether-lipid phospholipid	1-acyl-sn-glycerol-3-phosphate acyltransferase-like protein	2.3.1.23	237.m00680 ||Tb11.01.6800	8017.m00006 ||Tc00.1047053509157.60	LmjF32.1960
lipid metabolism ether-lipid phospholipid	dihydroxyacetone phosphate acyltransferase, possible	2.3.1.42	Tb04.2H8.550	4886.m00004	LmjF34.1090
lipid metabolism|folate metabolism|pteridine	pteridine reductase ?	1.5.1.33	228.m00311 ||Tb08.26N11.790	none	LmjF23.0270 |PTR1
lipid synthesis eukaryotic	3-oxoacyl-(acyl-carrier protein) reductase, probable		none	none	LmjF24.2030
oxidoreductase | lipid metabolism	putative electron transfer flavoprotein beta-subunit beta-etfflavoprotein small subunit		Tb11.03.0950	Tc00.1047053504109.160	LmjF25.0120

Table 6. Trypanosomatid enzymes involved in phopholipd metabolism

Enzyme name	**EC Number**	**GeneDB accession**		
		T. brucei	***T. cruzi***	***L. major***
1-acyl-sn-glycerol-3-phosphate acyltransferase	2.3.1.23	Tb11.01.6800	Tc00.1047053509157.60	LmjF32.1960
alkyldihydroxyacetonephosphate synthase	2.5.1.26	Tb06.28P18.170	Tc00.1047053505807.110	LmjF30.0120
cdp-diacylglycerol synthetase	2.7.7.41	Tb07.8P12.300	Tc00.1047053511237.40	LmjF26.1620
cholinephosphate cytidylyltransferase	2.7.7.15	Tb10.389.0730	Tc00.1047053509805.220	LmjF18.1330
DHAP acyltransferase	2.3.1.42	Tb04.2H8.550	Tc00.1047053504055.40	LmjF34.1090
Diacylglycerol cholinephosphotransferase	2.7.8.2	Tb10.6k15.1570	Tc00.1047053509791.150	LmjF36.5900
dihydroxyacetone phosphate acyltransferase	2.3.1.42	Tb04.2H8.550	Tc00.1047053504055.40	LmjF34.1090
ethanolamine-phosphate cytidylyltransferase	2.7.7.14	Tb11.01.5730	Tc00.1047053511727.120	LmjF32.0890
glycerophosphoryl diester phosphodiesterase	3.1.4.46	Tb11.01.0580	Tc00.1047053511071.100	LmjF36.5960
lysophospholipase	3.1.1.5	Tb08.11J15.160	Tc00.1047053511907.50	LmjF24.1840
myo-inositol monophosphatase	3.1.3.25	Tb05.26K5.720	Tc00.1047053507047.120	LmjF17.1390
phosphatidic acid phosphatase	3.1.3.4	Tb08.25E9.660	Tc00.1047053503809.110	LmjF19.1350
phosphatidylethanolamine N-methyltransferase	2.1.1.17	Absent	Absent	LmjF31.3120
Phosphatidylinositol-specific phospholipase	3.1.4.11	Tb11.02.3780	Tc00.1047053504149.160	LmjF35.0040
phosphatidylinositol synthase	2.7.8.11	Tb09.160.0530	Tc00.1047053510349.50	LmjF26.2480
Phosphatidylserine decarboxylase proenzyme	4.1.1.65	Tb09.211.1610	Absent	LmjF35.4590
phosphatidylserine synthase	2.7.8.8	Tb07.28B13.50	Tc00.1047053509937.30	LmjF14.1200
phosphoenolpyruvate mutase	5.4.2.9	Absent	Tc00.1047053511589.140	Absent
phospholipase A1	3.1.1.32	Tb927.1.4830	Absent	Absent
phospholipase A2	3.1.1.4	Tb09.211.3650	Tc00.1047053510743.60	LmjF35.3020
phospholipase C	3.1.4.11	Tb11.02.3780	Tc00.1047053504149.160	LmjF30.2950
phospholipase C	3.1.4.3	Absent	Absent	Absent
phospholipase C glycosylphosphatidylinositol-specific (GPI-PLC)	3.1.4.3	Tb927.2.6000	Tc00.1047053506617.70	Absent
phospholipase D	3.1.4.4	Absent	Absent	Absent

Table 7. Enzymes of the urea cycle and putrescine synthesis

Enzyme name	EC Number	GeneDB accession		
		T. brucei	T. cruzi	L. major
agmatinase	3.5.3.11	Tb08.26N11.490	Tc00.1047053510947.40	LmjF23.0070
arginase	3.5.3.1	Absent	Absent	LmjF35.1480
argininosuccinate lyase	4.3.2.1	Absent	Absent	Absent
argininosuccinate synthase	6.3.4.5	Absent	Absent	LmjF23.0260
carbamoyl-phosphate synthase (ammonia)	6.3.4.16	Tb05.6E7.720	Tc00.1047053508373.10	LmjF16.0590
ornithine carbamoyltransferase	2.1.3.3	Absent	Absent	Absent
ornithine decarboxylase	4.1.1.17	Tb11.01.5300	Absent	LmjF12.0280


Glycosome
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ATP
ADP
1
GlcN-6-P
11
Glc-6-P
P-5-P
H_2O
NH_3
CO_2
2
Fru-6-P
PPP
Man-6-P
12
P_i
3b
ATP
3a
ADP
Fru-1,6-P_2
4
5
DHAP
GAP
NADH
P_i
NAD^+
8
6
NAD
NADH
glycerol-3-P
G-1,3-P_2
9
ADP
ATP
AMP
10
ATP
ADP
fumarate
21
Succinate
H_2O
20
NADH
NAD
malate
19
oxaloacetate
ATP
18
CO_2
ADP
P_i
22
PEP
pyruvate
AMP + PP_i
ATP
Monosaccharides
25
Disaccharides
24
Polysaccharides
13
Man-1-P
14
GDP-Man
?
β-mannan
Glycerol
Triglycerides
G-1,3-P_2
ADP
ATP
7
3-PGA
15
2-PGA
16
PEP
CO_2
ADP
ATP
18
ADP
ATP
17
pyruvate
oxaloacetate
glutamate
23
α-ketoglutarate
Alanine
Cytosol

Fig 1

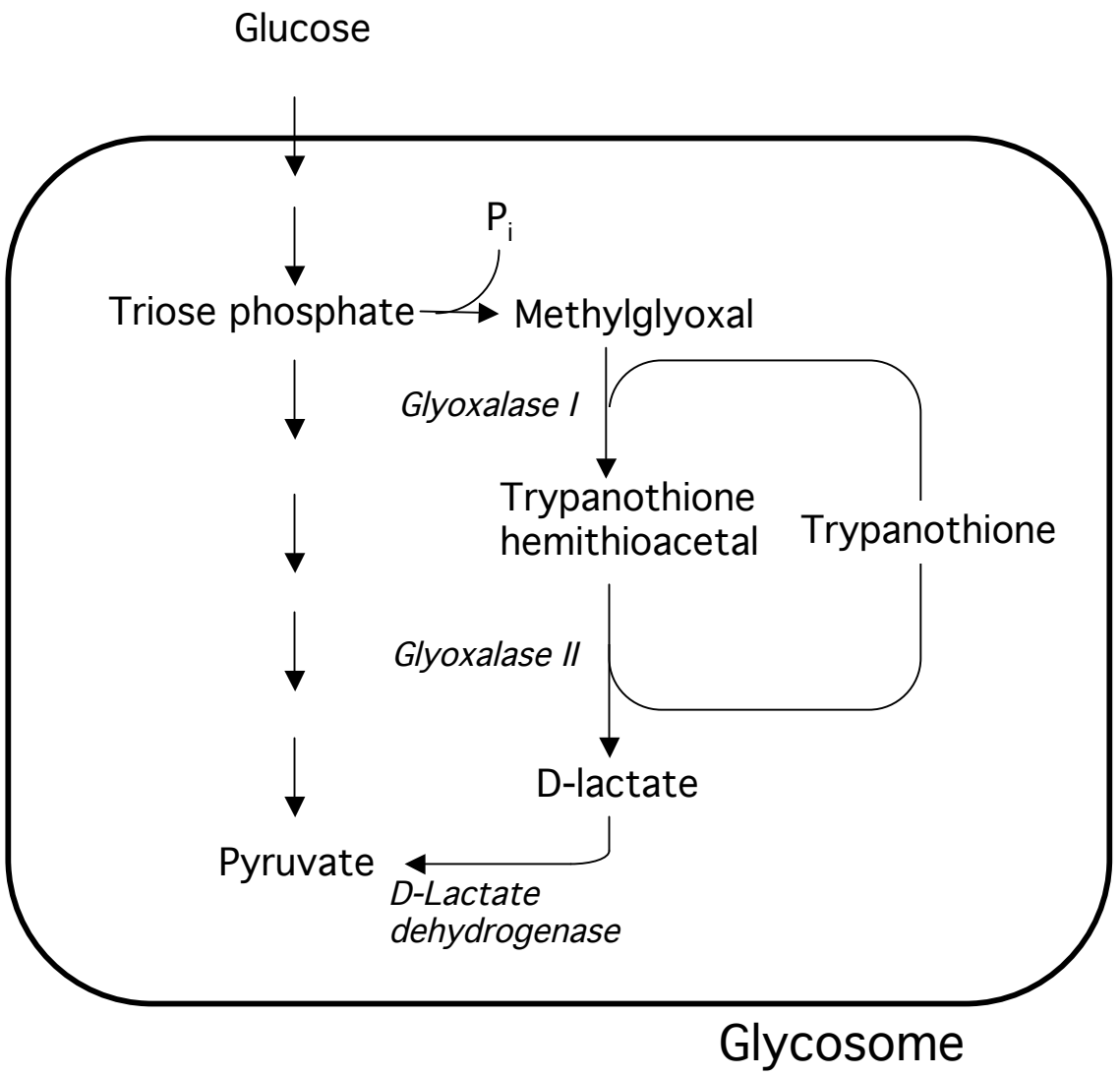
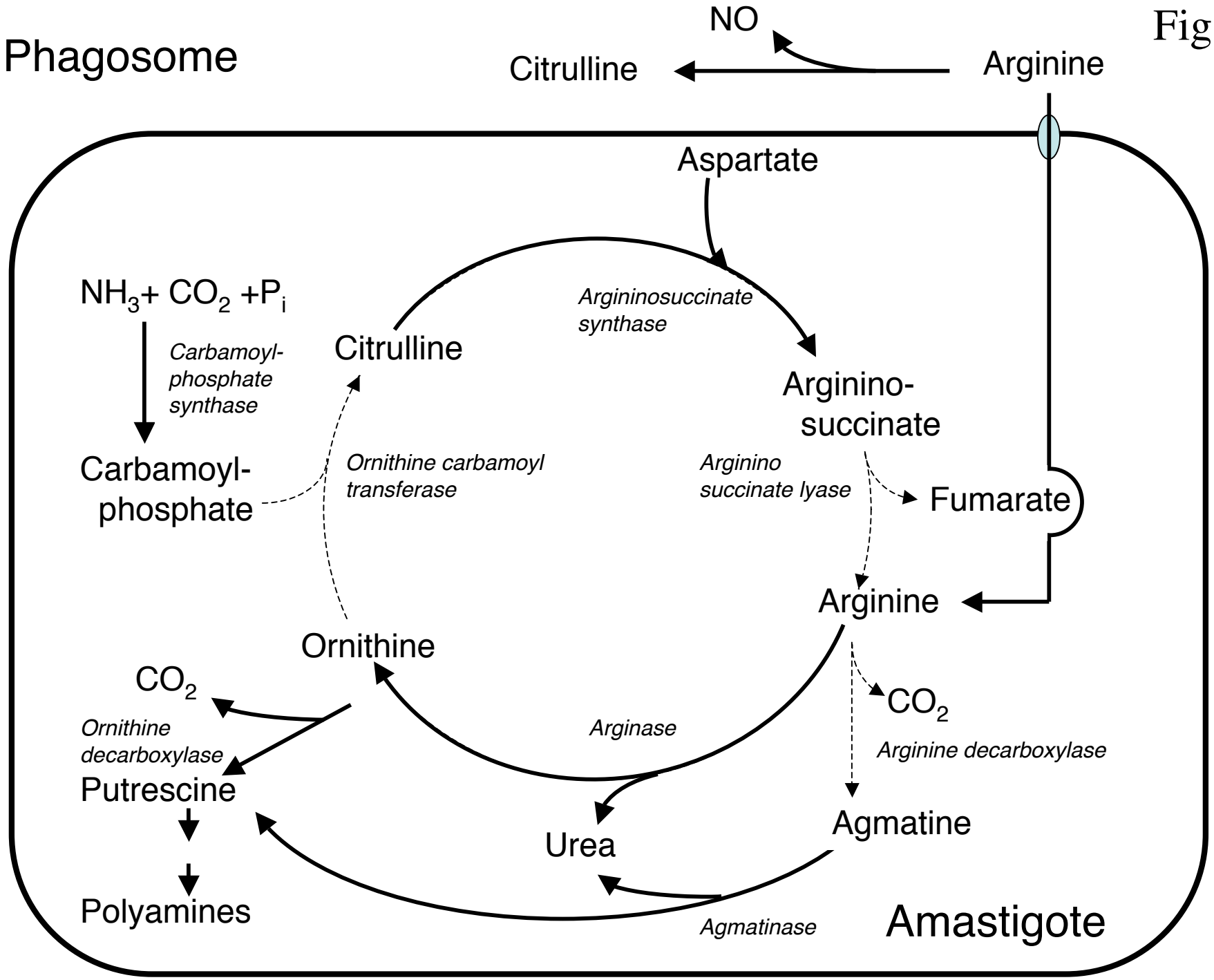

Glucose
P_i
Triose phosphate
Methylglyoxal
Glyoxalase I
Trypanothione hemithioacetal
Trypanothione
Glyoxalase II
D-lactate
Pyruvate
D-Lactate dehydrogenase
Glycosome

Fig 2

Fig 3


Phagosome
NO
Citrulline
Arginine
Aspartate
NH_3+ CO_2 +P_i
Carbamoyl-phosphate synthase
Carbamoyl-phosphate
Citrulline
Argininosuccinate synthase
Arginino-succinate
Ornithine carbamoyl transferase
Arginino succinate lyase
Fumarate
Arginine
Ornithine
CO_2
Ornithine decarboxylase
Putrescine
Polyamines
Arginase
Urea
CO_2
Arginine decarboxylase
Agmatine
Agmatinase
Amastigote