

Comparative genomics of trypanosome metabolism

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Abstract

Over the last fifty years, the metabolism of *Trypanosoma brucei* and *Trypanosoma cruzi* has been the subject of many extensive biochemical investigations, but the recent completion of two trypanosome genome sequencing projects now provides a more complete insight in the full metabolic capacities of these two trypanosomatids. The genes required for various pathways of carbohydrate metabolism, including glycolysis and the hexose-monophosphate pathway, are all present, but a comparison between African and American trypanosomes indicates that only *T. cruzi* contains a considerable number of genes encoding bacterial-type kinases with predicted specificity for various sugars, other than glucose. The enzymes encoded by these genes all contain targeting signals for import into the glycosomes, suggesting that in American trypanosomes the glycosomes have adapted to the breakdown of a wide variety of sugars. In *T. brucei* the carbohydrates that can provide carbon for energy metabolism are likely to be limited to glucose, fructose and mannose. No evidence in either trypanosome species was found for the presence of either a functional glyoxylate cycle or uric acid cycle. Both *Trypanosoma* species are capable of synthesising and oxidising fatty acids. Consistent with previous biochemical studies, the capacity of trypanosomes for general lipid synthesis is, in comparison with many parasitic protozoa, impressive. Most amino acids, apart from the aromatic ones, can be oxidised by the two organisms, but amino-acid synthesis is generally limited to the so-called non-essential amino acids. *T. cruzi*, but not *T. brucei*, is able to utilise histidine as an energy source. The assembly of the mitochondrial respiratory chain, which is required for oxidative phosphorylation and efficient metabolism of amino acid and fatty acid carbon sources, appears to be a balance between conserved and unique biochemical processes. Finally, the presence of numerous genes of bacterial ancestry indicates that horizontal gene transfer has played an important role in shaping the trypanosomatids metabolic capacities.

Introduction

In this chapter we consider the impact that completion of the *Trypanosoma brucei* and *T. cruzi* genome sequencing projects has had upon our understanding of metabolism in these two medically relevant trypanosomatids. Different niche environments, in both the mammalian host and their respective invertebrate vectors, are occupied by these two *Trypanosoma* species meaning that a comparative assessment of the metabolic strategies used by each parasite is particularly insightful. In comparison to several other parasitic microbial eukaryotes for which complete genome sequences are available (e.g. the malarial parasite *Plasmodium falciparum* (Gardner et al., 2002), another apicomplexan *Cryptosporidium hominis* (Xu et al., 2004), and the microsporidian parasite *Encephalitozoon cuniculi* (Katinka et al., 2001)) the trypanosomatids are much more experimentally tractable. Thus, even prior to the onset of the *Trypanosoma* genome sequencing projects, we had enjoyed detailed insight into the intermediary metabolism of these parasites. Notwithstanding this fact, the availability of genome sequences for *T. cruzi* and *T. brucei*, coupled to the use of modern molecular approaches to study gene function, have facilitated a significant reappraisal in our fundamental understanding of metabolism in both organisms.

Experimental studies of metabolism in *T. brucei* have focused almost exclusively on two life cycle stages: the bloodstream form encountered in the mammalian host and the procyclic stage, which is found in the tsetse midgut. Other important life-cycle stages, such as epimastigotes and metacyclic trypomastigotes, which are found in the salivary glands of infected tsetse flies, and short stumpy bloodstream forms cannot be maintained in culture. They cannot be isolated in sufficient quantities for detailed biochemical studies either. Metabolic studies of *T. cruzi* have, to date, mostly been carried out using epimastigotes, which are easily cultured. The study of amastigote metabolism can be complicated by the presence of the host cell. Although axenic amastigote model systems are available, we have, until recently, had far less insight into the metabolic strategies that become important to *T. cruzi* when it is in the mammalian host. The *in silico*-derived metabolic maps that can be drawn following genome sequencing provide an overall holistic view of metabolic potential, but cannot take into account the reality that many metabolic pathways are present or important in only some life-cycle stages. We shall illustrate how this is true for both *T. brucei* and *T. cruzi*, even though the predicted metabolic repertoires of both parasites are very similar. Thus, the *in silico* prediction of metabolic repertoires represents only the first stage within a bigger challenge of elucidating how such metabolism is regulated within the context of completing a complex life cycle within multiple environments. Our discussion focuses mainly on the metabolism of *T. brucei*, but where species-specific differences occur these are discussed in a comparative context.

From previous studies, it is well known that bloodstream form *T. brucei* relies exclusively on glycolysis for energy generation. Glucose and other hexose sugars, such as fructose and mannose, and under aerobic conditions also glycerol, are converted to pyruvate, which is excreted into the host's bloodstream as the sole end-product (van Hellemond et al., 2005). The first seven enzymes of the pathway are located inside

specialized microbodies of the peroxisome class called glycosomes (Opperdoes & Borst, 1977). Regeneration of glycolytic NAD^+ inside this organelle takes place by an NAD-linked glycerol-3-phosphate dehydrogenase converting dihydroxyacetone phosphate to glycerol-3-phosphate (Opperdoes et al., 1977). The latter intermediate metabolite is reoxidised by a mitochondrial cyanide-insensitive alternative oxidase. In the absence of molecular oxygen, a situation which African trypanosomes may frequently encounter in the interstitial spaces of the host, glycerol-3-phosphate cannot be re-oxidised and accumulates within the glycosomes. As a result the glycerol kinase reaction operates in the reverse direction and glycerol is excreted at the expense of pyruvate. The glycolytic end-product pyruvate is not metabolised further because many mitochondrial pathways, including pyruvate dehydrogenase, the tricarboxylic acid (TCA) cycle and the respiratory chain are all absent at this point in the life cycle. ATP synthase is present, but functions in the direction of ATP hydrolysis, rather than ATP production, contributing to the maintenance of a proton gradient across the inner mitochondrial membrane. This is necessary for the translocation of nuclear-encoded proteins into the mitochondrial matrix (Nolan & Voorheis, 1992; Schnauffer et al., 2005). Thus, under aerobic conditions the ATP yield is limited to two moles per mole of glucose consumed; under anaerobic conditions this is reduced to one mole. Due to the absence of TCA-cycle activity long slender bloodstream forms are incapable of oxidising amino acids or fatty acids. In contrast, procyclic (insect form) *T. brucei* express a full set of mitochondrial respiratory chain complexes, and all enzymes of the TCA cycle have been detected. However, despite this more elaborate metabolic potential, glucose metabolism in procyclic *T. brucei* remains highly fermentative: in addition to carbon dioxide, large amounts of succinate and acetate are excreted (e.g. van Weelden et al., 2005). In procyclic cells glycosomal NAD^+ can be reoxidised by a glycosomal malate dehydrogenase which reduces oxaloacetate to malate. Malate is then metabolised further in the glycosome through the intermediacy of fumarate to yield succinate (Besteiro et al., 2002). The latter is excreted. Surprisingly, although a complete cohort of TCA cycle enzymes is present in the procyclic mitochondrion pyruvate is not oxidised to carbon dioxide and water (van Weelden et al., 2003; van Weelden et al., 2005). After its decarboxylation, the resulting acetyl-CoA is converted to acetate which leads to the formation of an additional molecule of ATP (Bochud-Allemann & Schneider, 2002; Riviere et al., 2004). Although glucose is readily used by procyclic cultures as a preferred carbon source for energy metabolism, carbohydrates are widely considered to be available in only limited quantities, if at all, in the tsetse midgut. Under conditions of glucose limitation in culture, amino-acids, such as proline and threonine, provide alternative substrates for ATP production.

In culture, *T. cruzi* epimastigotes also prefer to use glucose over amino acids as an energy substrate (Alonso et al., 2001). Under aerobic conditions they produce, in addition to CO_2 , considerable amounts of succinate, L-alanine, and acetate (Cazzulo, 1992). Epimastigotes produce ammonia only after the glucose in the medium has been exhausted (Alonso et al., 2001). An axenic culture model suggests that *T. cruzi* amastigotes mostly use glycolytic metabolism for ATP production (Engel et al., 1987). Amastigotes also ferment glucose to succinate and acetate, but do not seem to excrete ammonia and have little need for the oxidation of amino acids.

Carbohydrate metabolism - new insights from the genome

All of the genes in *T. brucei* and most of the genes in *T. cruzi* that are necessary for the uptake and degradation of glucose via the glycolytic pathway were identified prior to the arrival of the trypanosome genome sequences (Opperdoes & Michels, 2001). We have also known for several years prior to the initiation of genome sequencing that in their different life cycle forms both trypanosome species express on their plasma membranes glucose transporters with kinetic properties that reflect the likely glucose concentration in the different niche environments occupied by each parasite (Tetaud et al., 1997). Thus, in the glucose-rich environment of the mammalian bloodstream African trypanosomes express a low affinity transporter with a high capacity for carbohydrate transport. On the other hand within the low glucose environments of the digestive tract in their respective invertebrate vectors *T. brucei* procyclics and *T. cruzi* epimastigotes express transporters with a higher affinity for glucose. The glucose transporter expressed by bloodstream *T. brucei* exerts considerable control over the rate of the glycolytic flux, and thus would be an excellent drug target (Bakker et al., 1999). Interestingly, the genome sequence of *T. cruzi* revealed the presence of a hexose-phosphate transporter (Tc00.1047053510661.100) (Berriman et al., 2005). The presence of this gene, which is absent from *Leishmania major* (another trypanosomatid for which a complete nuclear genome sequence is available) and *T. brucei*, can be correlated with the importance of glycolysis for energy generation in *T. cruzi* amastigotes, because although glucose metabolism is likely to be an important metabolic strategy for both the parasite and the host cell, the free glucose concentration in the host cell cytoplasm is probably low.

Although the identification of the genes encoding the glycolytic enzymes predated the onset of genome sequencing, the availability of complete, annotated genome sequences potentially provides some insight into the role that gene dosage can provide (**Table 1**). In our analysis copy numbers were initially determined using the BLASTp algorithm with a cut-off value of $1e^{-80}$ in order to only identify orthologous copies. In case certain obvious gene copies were not reported using this approach, a less stringent cut-off value was subsequently applied. From this analysis we found that *T. cruzi* has on average twice the number of gene copies that are present in *T. brucei*. However, many of these additional gene copies in *T. cruzi* are pseudogenes. Extending the analysis to other metabolic pathways (**Table 1**) reveals that genes coding for enzymes of carbohydrate metabolism and oxidant stress protection have on average a higher copy number than genes from other metabolic pathways. Gene dosage is possibly a mechanism for increasing the amount of enzyme required for specific reactions – in some instances deletion of one allele of a metabolic enzyme from the diploid genome of *T. brucei* yields a haploid insufficiency phenotype (Roper et al., 2005), lending further support for this particular gene dosage argument. Several glycolytic enzymes (hexokinase, aldolase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, fumarate reductase) plus the pyruvate and glucose transporters are encoded with a copy number greater than one, often as multiple tandem-linked gene copies. However, a particularly interesting example is the occurrence in *T. brucei* of five almost identical genes encoding glycosomal glycerol kinase, whereas in *T. cruzi* this enzyme is encoded by a single copy gene. In *T. cruzi* glycerol kinase can be utilized for the oxidation of glycerol derived from triglyceride metabolism, but in the African trypanosome a primary function in

bloodstream stages is in the endothermic production of glycerol as a glycolytic end-product under hypoxic or anaerobic conditions (Hammond & Bowman, 1980a, b).

Another way in which the trypanosomatid genome sequences can be interrogated is in the identification of the set of genes that encode enzymes containing either a PTS-1 or a PTS-2, which are the two well-conserved targeting signals that confer a glycosomal location upon many of the proteins in which they occur (Moyersoet al., 2004). In this way many additional aspects of glycosomal carbohydrate metabolism have been identified (**Figures 1 and 2, Table 1**) (Berriman et al., 2005; Opperdoes & Szikora, 2006). For instance, several bacterial-type sugar kinases (glucokinase, galactokinase and L-ribulokinase) are encoded in the *T. cruzi* genome, and all contain targeting signals for import into glycosomes. Similar orthologous genes are also present in the genome of *L. major*, where their presence can be readily correlated to the availability of a diverse array of sugars within the plant nectar upon which the sandfly vector of *Leishmania* often feeds. The biochemical demonstration of a *Leishmania* sucrase was reported some years ago (Blum & Opperdoes, 1994), but the information revealed by genome sequencing identifies an unexpected adaptation of glycosomes towards carbohydrate metabolism. The complete absence of sugar kinase genes from the *T. brucei* genome is explained by the more restricted range of sugars that are present in plasma, and which are therefore available to African trypanosomes during their life cycle (tsetse flies are obligate blood feeders). It will be interesting to determine whether the additional sugar kinases of *T. cruzi* are important for parasite fitness within the mammalian host since its reduviid bug vector is, like the tsetse fly, reported to be an obligate blood feeding arthropod.

In addition to glycolysis, *Trypanosoma* are capable of gluconeogenesis. At least in African trypanosomes this pathway is probably only functional in the insect stages, where external glucose availability is likely to be limited. Despite low glucose availability, glucose-6-phosphate is still required by *T. brucei* for the synthesis of polysaccharides and glycoproteins. TCA-cycle intermediates probably provide a major source of carbon precursors for gluconeogenesis (van Weelden et al., 2005). Genes essential for gluconeogenesis [i.e. phosphoenolpyruvate carboxykinase and fructose-1,6-bisphosphatase (Hannaert et al., 2003)], as well as genes involved in the synthesis of glycogen and other polysaccharides, are all present and some carry glycosomal targeting signals. Genes for two regulatory enzymes, 6-phosphofructo-2-kinase and fructose-2,6-bisphosphatase, are also present (Chevalier et al., 2005). However these enzymes are likely to be cytoplasmic as they lack canonical glycosomal targeting signals. These latter two enzymes are responsible for the formation and degradation of fructose-2,6-bisphosphate, which in many organisms is an allosteric regulator of phosphofructokinase. In trypanosomatids, however, fructose-2,6-bisphosphate is a potent regulator of the cytosolic enzyme pyruvate kinase (van Schaftingen et al., 1985). Intriguingly, the presence of a PTS in both phosphofructokinase and fructose-1,6-bisphosphatase, catalysing opposite reactions of the glycolytic and gluconeogenic pathways, respectively, suggest that both enzymes are present inside glycosomes and, therefore, should be strictly regulated. In other organisms, phosphofructokinase and fructose-1,6-bisphosphatase are regulated through phosphorylation and dephosphorylation, by a protein kinase and a protein phosphatase, respectively. The presence of two PTS-containing protein kinases

(Tb10.6k15.0770 and Tb10.389.0490) and a PTS-containing protein phosphatase (Tb04.1D20.130) provides tantalizing *in silico* evidence that activity regulation by (de)phosphorylation may take place inside the glycosome, too.

As part of its strategy for the detoxification of reactive oxygen species *T. cruzi*, but not *T. brucei*, contains an ascorbate-dependent peroxidase which is targeted to the endoplasmic reticulum (Wilkinson et al., 2002) suggesting that American trypanosomes are capable of scavenging or synthesizing ascorbate (vitamin c). In fact, analysis of the completed genome sequences reveals that both *T. brucei* and *T. cruzi* are capable of ascorbate synthesis using a biosynthetic pathway from a galactose precursor, similar to the pathway that occurs in plants (Wilkinson et al., 2005). Interestingly, the *Trypanosoma* arabinolactone oxidase carries a PTS, and in *T. brucei* epitope-tagged enzyme accumulates in glycosomes (Wilkinson et al., 2005). Moreover, phenotypic characterization of arabinolactone oxidase null bloodstream *T. brucei* mutants indicates that (i) intracellular ascorbate is likely to play a role in cell fitness, perhaps as a component of a sophisticated antioxidant defence system and (ii) that intracellular ascorbate accumulates as a consequence of both de novo synthesis and its acquisition from the external environment. Recent studies also indicate that *T. brucei* UDP-glucose epimerase, an essential enzyme required for galactose formation was cloned as a consequence of the genome sequencing project, is also a glycosomal enzyme (Roper et al., 2005).

Finally, trypanosomes contain a full set of candidate genes for all of the enzymes of the conventional pentose-phosphate pathway. Out of the seven enzymes encoded by these genes, three (6-phosphogluconate dehydrogenase, transaldolase and ribulose-phosphate-3-epimerase) have in *T. brucei* no detectable glycosomal targeting signal predicted, whereas in *T. cruzi* only one, transaldolase, lacks such a signal. The completed *Trypanosoma* genome sequences also encode sedoheptulose-1,7-bisphosphatase, which is better known as an enzyme of the Calvin cycle, but in trypanosomes is hypothesized to be involved in a modified pentose-phosphate pathway ((Hannaert et al., 2003); **Figure 2**). This enzyme also carries a PTS. Thus, taken in the context of earlier biochemical studies the possibility now emerges that a complete pentose-phosphate pathway may be operational in glycosomes (Cronin et al., 1989; Duffieux et al., 2000). Plausible functions of glycosomal pentose phosphate pathway activity may be to generate NADPH as a defence against intra-organellar reactive oxygen species and to provide ribose for pyrimidine biosynthesis (see below).

Curiosities of an aerobic mitochondrion with some fundamentally anaerobic traits

The mitochondrion is used constitutively for energy generation by *T. cruzi*, but the role of the *T. brucei* mitochondrion in energy generation is subject to strict stage-specific regulation (van Hellemond et al., 2005). Many of the genes required for mitochondrial energy generation are encoded in the nuclear genome. The major end-products of carbohydrate metabolism in procyclic *T. brucei* are acetate, succinate and carbon dioxide. A large proportion of the excreted succinate is produced in the glycosomes by NADH-dependent fumarate reductase, whereas acetate is produced in the mitochondrion from acetyl-CoA by the TCA-cycle enzyme succinyl-CoA ligase and an acetate:succinate CoA

transferase (ASCT) (Van Hellemond et al., 1998). Together, the latter two enzymes catalyse a cycle leading to the net production of acetate and one mole of ATP (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). In procyclic *T. brucei*, which lacks any capacity for anaerobic growth (van Weelden et al., 2003), the use of the ASCT cycle is probably essential for development in the tsetse midgut (Riviere et al., 2004). The molecular characterization of glycosomal fumarate reductase (Besteiro et al., 2002) and ASCT (Riviere et al., 2004) were both facilitated by the release of genome survey sequences prior to the annotation of the completed genome.

Even though the presence of a complete cohort of TCA cycle enzymes can be predicted from the *T. brucei* genome sequence, there is no evidence that, at least in culture, the TCA cycle is used for oxidation of acetyl-CoA to CO₂ (Besteiro et al., 2002; van Weelden et al., 2003; van Weelden et al., 2005). This suggests that a primary role of the TCA cycle for a parasite population expanding in the tsetse midgut is for the provision of precursors for biosynthetic pathways, such as gluconeogenesis. Such assertions are consistent with the likely low availability of glucose and other carbohydrates in the tsetse midgut. Of course, one cannot rule out that the TCA cycle is used for oxidation of acetyl-CoA at some other point during the transmission cycle of African trypanosomes through tsetse, but the recently published results based on biochemical investigations (Besteiro et al., 2002; van Weelden et al., 2003; van Weelden et al., 2005) provide an excellent illustration of how hypothetical metabolic maps produced as a consequence of genome sequencing can be misleading at the same time that they have the potential to be highly informative. Since the end-products of intermediary metabolism in other trypanosomatids, including *Leishmania* and *T. cruzi*, are characteristic of aerobic fermentation (Cazzulo, 1992), an important area for future research is to determine the role that TCA cycle activity plays, if any, in energy generation during different trypanosomatid life cycles.

The importance of the ASCT cycle and mitochondrial substrate level phosphorylation as a major, essential route for acetyl-CoA metabolism and ATP production in procyclic *T. brucei* (Bochud-Allemann & Schneider, 2002; Riviere et al., 2004) correlates with entry into the TCA cycle as only a minor route for acetyl-CoA metabolism – a build-up of citrate in aconitase null mutants indicates that condensation of acetyl-CoA with oxaloacetate, the first reaction of the TCA cycle catalysed by citrate synthase, does occur [(van Weelden et al., 2003); see a later discussion for how such acetyl-CoA, through the intermediacy of citrate, might be used for cytoplasmic fatty acid biosynthesis]. Yet, procyclic trypanosomes are, in addition to mitochondrial substrate level phosphorylation, also critically dependent upon the mitochondrial respiratory chain for growth (Bochud-Allemann & Schneider, 2002; van Weelden et al., 2003). In procyclic *T. brucei* the electrons which enter the respiratory chain come mostly from amino acid metabolism (**Figures 1 and 3**, and discussed in greater detail in a subsequent section within this chapter). The H⁺ gradient that is set up across the inner mitochondrial membrane as a consequence of electron transport to the terminal acceptor O₂ through the intermediacy of ubiquinone and cytochrome *c* as mobile electron carriers is used in two ways: (i) for

import of proteins synthesized on cytoplasmic ribosomes into the mitochondrial matrix, and (ii) by ATP synthase for generation of ATP from ADP and P_i. In the absence of an external carbohydrate source, procyclic trypanosomes are particularly sensitive to the addition of oligomycin, which is a classic inhibitor of ATP synthase (Lamour et al., 2005). However, in addition to the respiratory chain which uses O₂ as its final electron acceptor, the recent molecular characterisation of genes encoding soluble *T. brucei* mitochondrial fumarate reductases highlights a further route that could be potentially used for re-oxidation of NADH generated through carbohydrate or amino acid catabolism (Coustou et al., 2005). In the presence of glucose, approximately 30% of the succinate produced by cultured parasites is derived from mitochondrial fumarate reductase activity. It is important to remember that the trypanosome fumarate reductases are all NADH dependent and are not linked to ubiquinol oxidation, as occurs with membrane bound fumarate reductase in helminth mitochondria, for example (van Hellemond et al., 2003). Although the functional importance of trypanosome mitochondrial fumarate reductase activity for the transmission cycle through tsetse is not obvious, the use of fumarate as an electron sink can be seen as fundamentally an anaerobic trait. Perhaps then, although *T. brucei* is critically dependent on oxidative metabolism for growth, the identification of alternative routes for maintaining NAD⁺/NADH redox balance suggests that the parasite is suitably adapted to resist transient conditions of hypoxia or anoxia within the digestive tract of its insect vector. Since mitochondrial fumarate reductases are conserved in other trypanosomatids, perhaps this speculative paradigm holds true in these species, too.

The respiratory chain and oxidative phosphorylation

In many respects the trypanosome mitochondrial respiratory chain is similar to the which is described in many standard biochemistry text books. The identity of many conserved nuclear encoded components of the trypanosome NADH:ubiquinone oxidoreductase (complex I), succinate dehydrogenase (complex II), cytochrome *c* reductase (complex III), cytochrome *c* oxidase (complex IV), ATP synthase (complex V), 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, were reported recently (Allen et al., 2004; van Weelden et al., 2005). In addition to these components, a FAD-dependent glycerol-3-phosphate dehydrogenase is used for re-oxidation of cytoplasmic NADH, via the dihydroxyacetone-phosphate:glycerol-3-phosphate shuttle. In bloodstream *T. brucei* re-oxidation of cytoplasmic NADH is linked to an alternative plant-like oxidase that transfers electrons directly from ubiquinol to oxygen. The alternative oxidase is also expressed in procyclic *T. brucei* (Chaudhuri et al., 2002), but appears to be specific for *T. brucei*, since a homologue identified in *T. cruzi* lacks some residues essential for catalysis.

Several components of the respiratory chain complexes, and also several essential cytoplasmic and nuclear proteins which are conserved in all eukaryotes, bind iron-sulphur (Fe-S) clusters. The identification from the *T. brucei*, *T. cruzi*, and *Leishmania* genome databases of putative orthologues for mitochondrial cysteine desulfurase, the NifU scaffold protein, ferredoxin, ferredoxin reductase, frataxin, the sulphhydryl oxidase *erv1*,

and ISA homologues suggests that mitochondrial synthesis and export of Fe-S clusters occur through pathways that are similar to those found in yeast and animals. On the other hand, trypanosomes are auxotrophic for haem, the cofactor for mitochondrial *b*- and *c*-type cytochromes. This auxotrophy provides an interesting contrast with asexual blood stage malarial parasites which synthesise haem even though the interior of the host erythrocyte provides a potentially salvageable source of haem in the form of haemoglobin. The completion of the *Trypanosoma* genome projects confirms the absence of any known pathway for haem synthesis, although in the insect vector once the confines of the peritrophic matrix have been crossed, a salvageable source of haem for either American or African trypanosomes is not obvious.

Understanding how hydrophobic haem molecules are trafficked through the cytoplasm and into the mitochondrion where non-covalent association with *b*-type cytochrome and covalent attachment to cytochromes *c* and *c*₁ presumably occurs constitutes an interesting problem worthy of future attention. However, with the sequences of several trypanosomatid genomes at hand it was recently reported that there is no trace in either nuclear or mitochondrial genomes of genes encoding any component of the three known pathways by which *c*-type cytochromes are matured (Allen et al., 2004) – a striking contrast with the conserved pathway for Fe-S cluster biogenesis and the components of the oxidative phosphorylation apparatus which are all present. The absence of any known cytochrome *c* maturation machinery also correlates with the unique haem attachment to mitochondrial cytochromes *c* and *c*₁ in kinetoplastids and euglenids, where attachment occurs through a single thioether linkage between a vinyl group of the haem and the cysteine in a XXXCH haem binding site of the apocytochrome (Pettigrew et al., 1975; Mukai et al., 1989; Ambler et al., 1991; Priest & Hajduk, 1992). In all other *c*-type cytochromes haem attachment occurs through two thioether linkages between both vinyl groups of the haem and two cysteines in a CXXCH haem binding motif (where X is any amino acid except cysteine) (Allen et al., 2003). Studies using recombinant *T. brucei* cytochrome *c* indicate there is no reason why this cytochrome cannot accommodate the first cysteine within its haem-binding motif (Allen et al., 2004). The available biochemical evidence suggests single-cysteine haem attachment is the result of a positive evolutionary process, possibly linked to the environment of the mitochondrial intermembrane space, loss of a known ancestral cytochrome *c* maturation apparatus, or evolution/acquisition of a novel pathway for *c*-type cytochrome maturation.

Mitochondrial transporters

On the basis of genome analysis, *T. brucei* possesses an expected set of mitochondrial solute transporters. For both pyruvate, the end-product of glycolysis, and P_i there are specific mitochondrial carriers. Other solute transporters appear to be homologues of dicarboxylate carriers that have been described in other organisms. A tricarboxylate exchanger is also present. These dicarboxylate and tricarboxylate carriers are probably involved in transport of TCA cycle intermediates and of aspartate and glutamate across the inner mitochondrial membrane, although without appropriate biochemical analysis it is not possible to determine the substrate specificities of individual carrier proteins on a basis of sequence homology alone. An ATP/ADP exchanger is also present, and would

facilitate efficient export of newly synthesized ATP from the mitochondrial matrix into the cytoplasm.

ATP homeostasis – adenylate kinases and arginine kinases

Adenylate kinase catalyses the reversible transfer of γ -phosphate from either an ATP or GTP donor to the AMP acceptor, yielding two molecules of ADP or one ADP and one GDP, respectively. It is a ubiquitous and essential reaction, implicated in the local homeostasis of adenine nucleotide pools and in the delivery of energy from intracellular sites of synthesis to sites of consumption (Dzeja & Terzic, 2003). For instance, within skeletal muscle cytoplasmic adenylate kinase plays an essential role in managing intracellular energy economy (Janssen et al., 2000). An unexpected revelation from the trypanosomatid genome sequences was a surprisingly large number of *T. brucei* and *T. cruzi* adenylate kinase genes, which encode a number of distinct isoforms. Intracellular positioning of these isoforms explains the occurrence of this large gene family (Ginger et al., 2005): in addition to conserved cytoplasmic and mitochondrial activities (Opperdoes et al., 1981), there is a glycosomal adenylate kinase (containing a PTS-1), which is required by procyclic *T. brucei* in order to maintain an optimum growth rate in medium containing glucose (Ginger et al., 2005). However, perhaps the most important characteristic of the *T. brucei* adenylate kinase gene family is the anchoring within the flagellar axoneme and paraflagellar rod of three isoforms that possess unusually long (~50 amino acids) N-terminal extensions (Ginger et al., 2005). The extensions are required for these enzymes to partition into the flagellar compartment, and are also sufficient to confer flagellar localisation of a GFP reporter protein (Pullen et al., 2004). Moreover, the molecular characterisation of these isoforms and the biochemical identification of adenylate kinase activity in isolated flagella established the trypanosome flagellum as another organelle, distinct from cytoplasm, mitochondrion and glycosomes, with its own particular metabolic properties. Possible functional roles for the trypanosome flagellar adenylate kinases have been discussed previously, and may include a role in energy management that is analogous to the function of cytoplasmic adenylate kinase in mammalian muscle (Ginger et al., 2005).

The occurrence of arginine kinase genes in both *Trypanosoma* species is suggestive of further complexity with regard to the organisation and regulation of trypanosome energy metabolism. In *T. cruzi* one arginine kinase isoform is encoded by a gene present with a copy number of two per haploid genome, but in *T. brucei* the arginine kinase family has expanded, probably through tandem duplication, to yield three distinct isoforms that differ only with respect to their N- and C-termini and the intergenic sequences upstream and downstream of each gene. Thus, the genome sequence predicts that in addition to pathways for generating ATP through cytoplasmic and mitochondrial substrate level phosphorylation, as well as through oxidative phosphorylation, there is also a phosphagen kinase system that could function in energy homeostasis. However, such genomic-based observations should also be balanced against biochemical data, which indicated that the measurable arginine kinase activity of *T. cruzi* epimastigotes and *T. brucei* procyclics is much lower than the activity that can be measured in animal tissues where phosphagen kinase systems, such as those catalysed by creatine kinase and arginine kinase, provide a significant ATP buffering capacity in tissues that experience

high or fluctuating energy requirements (Pereira et al., 2000; Alonso et al., 2001; Pereira et al., 2002). Yet, it is also true that limitations in being able to accurately replicate in culture the environmental conditions found within host and vector compound attempts to elucidate the functional role of presently cryptic enzymes such as arginine kinase. It is perhaps interesting that the *T. brucei* arginine kinase with a possible PTS-1 is indeed glycosomal and is moreover expressed at significantly higher levels in long slender bloodstream trypanosomes than it is in procyclic trypanosomes (MLG and C.A. Pereira, unpublished observations).

Amino-acid metabolism **(i) catabolism**

In the absence of carbohydrates, amino acids (particularly proline, threonine and glutamic acid (Coustou et al., 2003)) may serve as important energy substrates for *T. brucei* in the midgut of the tsetse fly vector (**Figure 3**). L-Proline, the most abundant amino acid in the midgut and haemolymph of the tsetse fly, is oxidized to glutamate via mitochondrial delta-1-pyrroline-5-carboxylate dehydrogenase and pyrroline-5-carboxylate synthetase. *T. cruzi* is additionally capable of metabolising D-proline, as well as L-proline, since it also contains a putative proline racemase. In both *Trypanosoma* species the glutamic acid which results from (D,L)-proline degradation is deaminated by a mitochondrial glutamate dehydrogenase and then converted to succinate by TCA cycle enzymes (see above). However, in *T. cruzi* there are four genes involved in the conversion of histidine to glutamic acid, which were not detected in *T. brucei*. Comparative proteomic analysis of *T. cruzi* epimastigotes, metacyclic trypomastigotes, amastigotes, and bloodstream trypomastigotes recently revealed that the first two enzymes of this pathway, histidine ammonia-lyase and urocanate hydratase, are readily detected within the epimastigote and metacyclic proteomes, but were nearly undetectable in the mammalian amastigote and trypomastigote stages (Atwood et al., 2005). Since histidine is an abundant amino acid in both the excreta and haemolymph of the *Rhodnius prolixus*, this suggests a special case of adaptation of *T. cruzi* to its insect vector.

Branched chain amino acid metabolism has been the subject of several studies in *Leishmania* and *T. cruzi* (Montamat et al., 1987; Blum, 1991; Ginger et al., 1999; Ginger et al., 2000; Ginger et al., 2001). In animals and plants branched chain amino acid metabolism is a mitochondrial pathway and in trypanosomatids metabolism of these ketogenic amino acids provides substrates for the TCA and ASCT cycles. Interestingly, although there is biochemical evidence documenting branched chain amino acid metabolism in *T. cruzi* (Montamat et al., 1987; Ginger et al., 2000), no genes encoding putative branched chain amino-acid aminotransferases were evident in the *T. cruzi* genome. 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 the trypanosomatid sterol biosynthetic pathway (Ginger et al., 2001). The mevalonate pathway is the sole route used for isoprenoid biosynthesis in Trypanosomatids, but a direct route from leucine into this pathway, which is a major route for sterol biosynthesis, at least in culture, for several

Leishmania species and in other trypanosomatids (Ginger et al., 2001; 2000), has never previously been observed to operate in any other eukaryote. One explanation for why incorporation of leucine into isoprenoids by other eukaryotes occurs only after metabolism of the amino acid to acetyl-CoA is differential compartmentalisation of the catabolic leucine and biosynthetic isoprenoid pathways. Leucine catabolism is a mitochondrial pathway, whereas the reactions leading to mevalonate and thence isopentenyl diphosphate formation occur collectively in the cytoplasm, on the cytoplasmic face of the endoplasmic reticulum and, in some eukaryotes, within peroxisomes. Movement of acetyl-CoA from mitochondria into the cytoplasm often requires, as we will discuss in the subsequent section on trypanosome fatty acid biosynthesis, the operation of shuttle systems. 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). One can anticipate that the mitochondrion is a likely sub-cellular location for trypanosomatid leucine catabolism, too. Thus, the direct incorporation of leucine into sterols by trypanosomatids is likely to arise as a consequence of the unusual, possibly unique, occurrence of HMG-CoA reductase within the mitochondrial matrix, which is also probably the intracellular site of leucine degradation.

Most of the genes of the classical pathways for aromatic amino-acid oxidation are missing. The only gene for tryptophan metabolism detected in *T. brucei* and *T. cruzi* was one that is predicted to encode kynureninase, but this enzyme also plays a role in the biosynthesis of NAD co-factors. Some genes from the anaerobic phenylalanine degradation pathway are present, and in *T. cruzi* tyrosine can be converted to 4-hydroxyphenylpyruvate by two enzymes, a true tyrosine aminotransferase, probably also acting on phenylalanine and tryptophan, and a broad specificity aspartate aminotransferase (Tc00.1047053503679.10) (Vernal et al., 1998). While a canonical tyrosine aminotransferase gene could not be detected in *T. brucei*, a gene encoding the aspartate aminotransferase is present. Thus, both organisms should be able to convert aromatic amino acids to their corresponding ketoacids. In *T. cruzi* these ketoacids can be reduced by an aromatic hydroxyacid dehydrogenase, which probably arose by a moderate number of point mutations from an ancestral cytosolic malate dehydrogenase (Vernal et al., 2002). This enzyme, which now acts on various aromatic ketoacids rather than on oxaloacetate, is known as aromatic alpha-hydroxyacid dehydrogenase. It has been postulated that this enzyme fulfils an important role in the re-oxidation of cytoplasmic NADH. Orthologues of this malate dehydrogenase-like protein were also found in *T. brucei*, but since the *T. brucei* enzyme is not expressed in the bloodstream form (Aranda et al., 2006) it provides a plausible explanation for the accumulation and excretion aromatic ketoacids, in animals infected with *T. evansi*, a closely related trypanosome species (El Sawalhy et al., 1998). No enzymes of the classical pathway converting tyrosine to fumarate and acetoacetate were detected in any of the trypanosomatids. A functional urea cycle is also missing, and explains the excretion of ammonia as a product of trypanosomatid amino acid metabolism. In *T. brucei* ornithine is decarboxylated to putrescine for polyamine biosynthesis, but ornithine decarboxylase is not present in *T. cruzi*. Genes encoding arginase and agmatinase-like proteins are present, but arginine decarboxylase was not found with a BlastP e value of 1e-5. The apparent absence of the

capacity to form putrescine is in good agreement with the proposal that *T. cruzi* is a polyamine auxotroph, able to salvage spermidine and other polyamines from the medium (Carrillo et al., 2003; Aryanayagam et al., 2003).

(ii) synthesis

The capacity for the synthesis of amino acids by *T. brucei* and *T. cruzi* is limited to a few non-essential ones (**Figure 3**). The gene for D-3-phosphoglycerate dehydrogenase, the first enzyme committed to the synthesis of serine seems to be absent from *T. brucei*, while a gene for a phosphoserine phosphatase homologue could be present. Cysteine can be produced either from homocysteine by the trans-sulphuration pathway, but contrary to the situation in *T. cruzi* de novo synthesis from serine does not seem to be possible in *T. brucei*. In the latter the capacity for the interconversion of glycine to serine by a serine hydroxymethyl transferase is also absent. 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. However, *T. brucei* does not appear to be able to metabolise asparagine because an asparaginase gene does not appear to be present. Glutamate and glutamine can be formed from 2-ketoglutarate. Genes encoding enzymes involved in the synthesis of proline, such as gamma-glutamyl kinase and gamma-glutamyl phosphate reductase, or a gene encoding pyrroline-5-carboxylate synthetase, could not be detected in *T. brucei*, although the synthetase is present in *T. cruzi*. However, Δ -1-pyrroline-5-carboxylate can be reduced to proline by a Δ -1-pyrroline-5-carboxylate reductase for which the gene was found in both African and American *Trypanosoma* species. Pathways for the synthesis of threonine and methionine appear to be partially present. A homoserine dehydrogenase gene was not found in the *T. brucei* genome. A cystathionine β -lyase gene was also not present. However, methionine can be predicted to be salvagable from homocysteine in African trypanosomes, as is the case in *T. cruzi*. There is no capacity for the biosynthesis of aromatic amino acids (phenylalanine, tyrosine or tryptophan), branched amino acids (leucine, isoleucine and valine), lysine or histidine.

Lipid metabolism

In many ways, the arrival of complete genome sequences provides a reminder of some metabolic pathways that had not been studied in detail previously. Yet, it is also true that in many other aspects of lipid metabolism, notably isoprenoid biosynthesis and glycosylphosphatidylinositol anchor assembly, significant progress has been made and these pathways represent attractive targets for drug development in *T. cruzi* and *T. brucei*, respectively (Gelb et al., 2003; Roberts et al., 2003; Smith et al., 2004).

(i) lipids as carbon sources for energy generation and biosynthetic pathways

Although bloodstream *T. brucei* has no capacity for fatty acid oxidation, biochemical data based on measurements of enzyme activities suggested that tsetse-stage trypanosomes were capable of using fatty acids as a carbon source for energy generation (Wiemer et al., 1996), and this early conclusion is now supported by the presence of the appropriate genes within the *T. brucei* genome (see below). However, despite the characterization of appropriate enzyme activities no formal demonstration of fatty acid

oxidation using isotopically labelled precursors has been published. Additionally, no significant storage capacity for fatty acids has been reported in African trypanosomes. Similarly, direct evidence for the use of fatty acids as possible energy precursors during the life cycle of American trypanosomes had, until very recently, not been found. Some gaps in our understanding have, at least partially, been redressed as a consequence of the same proteomic analysis that provided evidence for an up-regulation of histidine metabolism during insect stages of the *T. cruzi* life cycle (Atwood et al., 2005, discussed earlier). Thus, a new metabolic model suggests that during the intracellular stages of the life cycle, when the parasite is found within host cell phagosomes - *i.e.* following cell invasion, but before escape into the host cytoplasm, where sugars or hexose phosphates can be acquired for energy metabolism - the capacity for β -oxidation of fatty acids is up-regulated. This result provides an interesting comparison with several pathogens, including *Leishmania*, that occupy phagolysosomal compartments inside mammalian macrophages (Lorenz & Fink, 2002; Lorenz et al., 2004; Munoz-Elias & McKinney, 2005). For many pathogens that thrive in this seemingly rather inhospitable niche, fatty acid metabolism appears to be critical for viability and virulence. Yet, the arrival of trypanosomatid genome sequences perhaps provides more questions than answers regarding the possible presence of another key pathway of lipid metabolism, the glyoxylate cycle.

The glyoxylate cycle is found in plants and many micro-organisms. This pathway is often discussed in the context of fatty acid metabolism because under conditions of nutritional stress, when carbohydrates and many amino acids are available in limited supply, it provides a route through which the C_2 intermediate acetyl-CoA can be assimilated to provide the gluconeogenic precursor phosphoenolpyruvate. Several biochemical studies suggested that the functional glyoxylate cycle was used by several species of *Leishmania* for the assimilation of acetyl CoA, produced by amino-acid and fatty acid oxidation, into carbohydrates (Simon et al., 1978; Keegan & Blum, 1993). However, these observations, based on the detection of the key enzyme activities catalysed by isocitrate lyase and malate synthase, were not replicated by another group (Mottram & Coombs, 1985). Additionally, although the incorporation of radiolabelled acetate into the carbohydrate polymer mannan is suggestive of a functional glyoxylate cycle (Keegan & Blum, 1993), the reported labelling pattern is also open to alternative interpretations since the two carbons of acetyl-CoA are not lost as CO_2 during the first turn of the TCA cycle following condensation with oxaloacetate. In agreement with the findings of Coombs and Mottram, the complete genome sequences of *T. brucei*, *T. cruzi*, and *L. major* also fail to provide any evidence for the presence of a classical glyoxylate cycle, suggesting that if glyoxylate cycle activity is present in the Trypanosomatidae the pathway may have arisen following changes in the substrate specificity of enzymes originally used for other purposes. Of course, there are plenty of examples of pathways that have either arisen or been retained following such evolutionary changes (Wu et al., 1999; van Hellemond et al., 2003; Madern et al., 2004). Since all of the Trypanosomatidae are considered to occupy environments, for at least part of their life cycles, that are limited in carbohydrate availability, it remains difficult to determine unequivocally whether a glyoxylate cycle is present or absent.

(ii) Post-genomic reconstruction of pathways for lipid metabolism in trypanosomes

(a) oxidation and synthesis of fatty acids

A full set of genes encoding the enzymes of β -oxidation confirms earlier reports that *T. brucei* would be able to oxidise fatty acids (Dixon et al., 1971). Four fatty acyl-CoA synthetases have been described previously, each displaying different chain-length specificities (Jiang *et al.*, 2000; Jiang & Englund, 2001) and in *T. cruzi* and *L. major* there are also multiple acyl-CoA dehydrogenases, each probably with a different chain length specificity as well. However, in *T. brucei* this capacity seems to be limited since only one such dehydrogenase was identified. Most of these genes encode predicted mitochondrial transit sequences, rendering the mitochondrion the most likely compartment for fatty acid oxidation. However, some of the enzymes also carry a putative PTS, suggesting that also glycosomes could be involved. The presence of genes encoding 3,2-trans-enoyl-CoA isomerase and 2,4 dienoyl-CoA reductase indicates that trypanosomes are also able to cope with the metabolism of unsaturated fatty acids.

Like the other trypanosomatids that have been analysed, procyclic *T. brucei* is able to synthesise fatty acids from suitable radiolabelled precursors, but in *T. brucei* bloodstream forms the sole end-product of the fatty acid biosynthetic pathway is myristic acid which is required for incorporation into the GPI-anchor of the VSG (Morita et al., 2000). In bloodstream stage parasites the fatty acyl chains required for phospholipid assembly (see below) are scavenged from the host, but the availability of host myristic acid may be insufficient to satisfy the parasite's special requirement for this particular fatty acid (Paul et al., 2001). Classically, fatty acid biosynthesis is a cytoplasmic pathway in eukaryotes, and the C_2 units that are used for synthesis of the fatty acyl chain often derive from mitochondrial acetyl-CoA. Thus, in the mitochondrion citrate is produced as a product of the reaction catalysed by citrate synthase (**Figure 2**) and is exported from the mitochondrial matrix into the cytoplasm using a tricarboxylate carrier, where it is cleaved by citrate lyase into oxaloacetate and acetyl CoA. The latter is then converted to malonyl CoA by an acetyl-CoA carboxylase and incorporated into the fatty-acid biosynthetic pathway. Although the intracellular site(s) of fatty acid biosynthesis in trypanosomatids have not been determined, the analysis of the complete *T. brucei* genome sequence reveals that the components required for export of citrate into the cytoplasm and a putative cytoplasmic citrate lyase are all present (van Weelden et al., 2005). Alternatively, the major end-product of energy metabolism in procyclic *T. brucei* is acetate which is produced in the mitochondrion by the ASCT and excreted from the cell. Since radioactive acetate species are readily incorporated into fatty acids by cultured trypanosomes (and other trypanosomatids), it is conceivable that some of the acetate produced in the mitochondrion finds its way into the fatty acid biosynthetic pathway rather than being excreted from the cell. A putative acetyl-CoA synthetase which would be required for acetate incorporation in fatty acids is also present in the *T. brucei* genome. Plenty of questions remain unanswered regarding the role of *T. brucei* TCA cycle enzymes during the tsetse transmission, but within the context of the natural life cycle (rather than during *in vitro* culture) it is conceivable that the relative contributions made to intracellular acetyl-CoA pools by pathways that make use of the ASCT cycle or citrate

lyase are determined by the relative flux of acetyl-CoA into the reactions catalysed by citrate synthase and ASCT, respectively (**Figure 1**). Looking further at the co-factor requirements for a putative cytoplasmic fatty acid biosynthetic pathway, we can use the available genome data to predict that NADPH can be formed by the conversion of oxaloacetate to malate by a cytosolic malate dehydrogenase and then to pyruvate by a cytosolic NADP-linked malic enzyme (van Weelden et al., 2005).

A combination of comparative genomics and biochemical studies indicates that the pathway for trypanosome fatty acid biosynthesis is also most unusual. There is no evidence for the presence of a cytoplasmic type I fatty acid synthase, which is a multifunctional enzyme used for fatty acid biosynthesis in many eukaryotes including mammals. Instead, four elongase genes seem to be responsible for the *de novo* synthesis of fatty acids (Paul Englund, personal communication). In mammals and yeast, elongases function to extend, via CoA-linked intermediates in a stepwise fashion, the end product (usually palmitate or stearate) of cytoplasmic type I fatty acid synthesis, but in *T. brucei* these elongases function in extending butyryl-CoA stepwise to myristate or stearate ((Paul et al., 2001); Englund P, personal communication). Similar elongases are present in the *T. cruzi* genome, too. The trypanosomatid genome sequences also predict the presence of a type II fatty acid synthesis system. Based on the presence of putative mitochondrial transit peptides this is most likely a mitochondrial pathway and could be used for synthesis of lipoic acid which is a necessary co-factor for pyruvate dehydrogenase, 2-ketoglutarate dehydrogenase and branched-chain ketoacid dehydrogenase. Type II fatty acid biosynthesis differs from the type I system of fatty acid biosynthesis in that the requisite enzyme activities are found on separate enzymes, rather than as part of a single multifunctional polypeptide. Acyl carrier protein (ACP), keto-acyl (ACP) synthase, and keto-acyl (ACP) reductase, homologous to Type II enzymes from bacteria and plant chloroplasts, were all readily identified. However acetyl-CoA:ACP transacylase and alpha-hydroxyacyl-ACP hydratase were not identified (Van Weelden et al. 2005).

(b) 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 trypanosomatids have been the subject of many biochemical studies that predated the arrival of the trypanosomatid genome sequences and have recently been reviewed in detail elsewhere (Roberts et al., 2003; Gelb et al., 2003). 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.

(c) synthesis of phospholipids

The first steps in the synthesis of phosphatidic acid, the intermediate common to triacylglycerol and glycerophospholipids biosynthesis, takes place inside glycosomes. Glycerol 3-phosphate (G3P) can be formed by reduction of dihydroxyacetone-phosphate (DHAP), which is catalysed by glycosomal glycerol-3-phosphate dehydrogenase (G3PDH), or by the phosphorylation of glycerol using a glycosomal glycerol kinase. Subsequently, either DHAP or G3P undergo an initial acylation reaction that uses a fatty

acyl CoA molecule as acyl group donor. Only one gene encoding an appropriate putative acyltransferase, a glycosomal DHAP acyltransferase, was evident in the *T. brucei* genome. This enzyme, which has been shown to be involved in the synthesis of ether lipids (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 trypanosome genome. Thus, it cannot be excluded that the glycosomal acyltransferase which was identified (Tb04.2H8.550 / Tc00.1047053504055.40) has a wide substrate specificity accepting both DHAP and G3P as acyl acceptor. A second, subsequent acyltransferase reaction is catalysed by lysophospholipid acyltransferase, or 1-acyl-sn-glycerol-3-phosphate acyltransferase, and is present in both *T. brucei* and *T. cruzi*. This enzyme, however, lacks a PTS and therefore one might anticipate 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 choline phosphate 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, however the latter enzyme was detected only in *L. major*, but not in *T. brucei* and in *T. cruzi*. Finally, acidic phospholipids can be formed from phosphatidic acid after their reaction with CTP to form CDP-diacylglycerols. Both phosphatidylserine and phosphatidylinositol can be formed from the latter family of compounds since the corresponding phosphatidylserine and phosphatidylinositol synthases are present. In *T. brucei*, but not in *T. cruzi*, phosphatidylserine can be converted to phosphatidylethanolamine by a phosphatidylserine decarboxylase. The latter enzyme may be mitochondrial because it carries a predicted mitochondrial transit peptide.

(d) phosphonolipids

T. cruzi synthesizes 2-aminoethylphosphonate (AEP or ciliatine) which is incorporated into macromolecules such as phosphonolipids and GPI molecules (Sarkar et al., 2003). This pathway, which utilises phosphoenolpyruvate as substrate, comprises the enzymes phosphoenolpyruvate mutase, phosphonopyruvate decarboxylase and 2-aminoethylphosphonate:pyruvate transaminase, all of which were detected in *T. cruzi*. Only the decarboxylase was found in the genome of *T. brucei*, and this is therefore in agreement with the absence of phosphonolipids from this trypanosomatid.

(e) ether phospholipids

Ether lipids are another significant constituent of trypanosomatid membranes. The first steps of the synthesis of ether lipids are shared with those of phospholipids and thus take place inside glycosomes (Heise & Opperdoes, 1997). The ether-lipid precursor acyl-DHAP is formed by a glycosomal DHAP-acyltransferase. The acyl moiety of acyl-DHAP is then exchanged with a fatty alcohol in a reaction catalysed by a glycosomal alkyl

DHAP synthase - the enzymes of *T. brucei* and *T. cruzi* both carry a PTS1. Alkyl-DHAP is then reduced to alkyl-G3P by the same alkyl DHAP synthase and exported to the cytosol where it undergoes conversion to alkyl phospholipids, probably by the same set of enzymes as those that act upon acyl-G3P to form normal phospholipids (see above).

Purine salvage and pyrimidine synthesis

Several studies reported that in common with other obligate parasitic protozoa the Trypanosomatidae are incapable of de novo purine synthesis (reviewed in Marr and Berens, 1983). This is now 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 *Trypanosoma* genomes, 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 glycosomal targeting signals confirming earlier claims that some of these enzymes 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 *T. brucei* genome these genes, except for the dihydroorotase, are all present on chromosome 5 and tandemly linked (**Table 1G**). In mammals the first three enzyme activities occur as a multifunctional protein that arose as a consequence of gene fusion. In trypanosomes 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 a bifunctional 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 particular 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 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).

Protection against oxidant stress

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 first step in polyamine biosynthesis is carried out by ornithine decarboxylase and this enzyme is the target for the drug difluoromethylornithine (eflornithine) in human sleeping sickness (Phillips et al., 1987). It is present in *T. brucei* but not *T. cruzi* (**Figure 3**). 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 genes coding for an agmatinase-like protein and an arginase are present, an arginine

decarboxylase was not found with a BlastP e value of 1e-5. This is in good agreement with the proposal that *T. cruzi* is a polyamine auxotroph, able to salvage spermidine from the medium (Carrillo et al., 2003). *T. cruzi* also differs in being capable of synthesising spermine as well as spermidine (Figure 3).

Cysteine can be produced from homocysteine by the trans-sulphuration pathway present in all three organisms. De novo synthesis from serine appears possible in *T. cruzi*, but not in *T. brucei* (**Figure 3**). The enzyme responsible for the conversion, cysteine synthase, is a typical plant-like enzyme, further only found in cyanobacteria, algae and plants. *T. cruzi* can also interconvert glycine and serine via serine hydroxymethyl transferase, but this capacity is not present in *T. brucei*. Methionine (as decarboxylated AdoMet), with cysteine, glutamate and glycine are the precursor amino acids for glutathione (**Figure 3**). 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. *T. cruzi* is unable to synthesize putrescine (see above), but salvages spermine, to incorporate this into trypanothione (Ariyanayagam et al., 2003). Synthesis of glutathione, polyamines and trypanothione have all been demonstrated to be essential for survival in *T. brucei* (Huyng et al., 2003; Li et al., 1998; Comini et al., 2004). Interestingly, not only trypanothione reductase, but also the enzymes responsible for its synthesis, glutathionylspermidine synthase and trypanothione synthetase, are of bacterial ancestry and have been acquired by events of lateral gene transfer. This could explain the unique nature of the oxidant stress protection mechanisms in trypanosomatids.

Trypanothione is essential for the defence against chemical and oxidant stress in trypanosomatids. It is also the target for trivalent arsenical and 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. Homologues of glyoxalase I and II are present in *T. cruzi*, whereas *T. brucei* apparently lacks glyoxalase I, although it does have a glyoxalase II (Irsch and Krauth-Siegel, 2004). The first enzyme of the pathway, methylglyoxal synthase, is absent from both organisms, although this is not surprising since in trypanosomes methylglyoxal is thought to be produced spontaneously from the triose phosphates produced in glycolysis.

Superoxide dismutase (SOD) is another essential part of the oxygen defense mechanism. It protects all obligate and facultative aerobic organisms from oxygen toxicity and damage. Trypanosomes each have four superoxide dismutases (SODA, two SODBs and an SOD-C). They are all of the iron type (Dufernez et al., 2006). 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., 2006). SODA and C are putative mitochondrial isoenzymes and the SODBs are glycosomal and cytoplasmic, respectively. There is no evidence for the presence of either a Mn-dependent enzyme (Dufernez et al., 2006) or Cu/Zn-superoxide dismutase. All pathogenic trypanosomatids lack catalase. They lack selenium-dependent peroxidases, too; 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. The latter has been shown to be a validated drug target (Augustyns et al., 2001).

A metabolism shaped by lateral gene transfer.

Phylogenetic tree reconstruction provides suggestive evidence that a plethora of genes involved in the core metabolism of trypanosomatids were acquired by lateral transfer from both bacteria and viruses (**Table 2**). Many genes coding for enzymes of the glycolytic pathway seem to be of prokaryotic origin, or show high affinity to their homologues from plants and algae. Indeed, the characterisation of a number of genes encoding enzymes with plant-like characteristics facilitated some debate over whether trypanosomatids ever harboured a plastid at some point during their evolution (El-Sayed et al. 2005; Hannaert et al. 2003; Leander 2004; Rogers and Keeling 2004). Although there is no ultrastructural evidence for any such hypothetical endosymbiosis, unlike the presence of a relic plastid (the apicoplast) in some apicomplexan parasites, it is intriguing that the trypanosomatid genomes reveal the presence of a gene encoding a hypothetical protein (Tb06.2N9.620, Tc00.1047053503909.50) for which there are only homologues in cyanobacteria and in chloroplast-bearing eukaryotes, such as green plants and algae. Within the extant Trypanosomatidae a small group possesses a bacterial endosymbiont, which relieves certain nutritional autotrophies that are placed upon other trypanosomatids. This endosymbiosis therefore provides evidence for a phagotrophic past, which according to Doolittle's "you are what you eat hypothesis" would have provided the opportunity for central metabolic processes to have been sculpted through lateral gene transfer.

In some instances lateral gene transfer is reflected in species-specific occurrence of enzymes, which potentially improve parasite fitness within particular niche environments (Berriman et al., 2005). A prime example that we mentioned earlier is the presence in *T. cruzi*, probably inside glycosomes, of sugar kinases which are specific for sugars other than glucose, and likely reflects an early adaptation of trypanosomatids to the plant-derived sugar-rich diets of their insect hosts. The absence of similar orthologous genes from *T. brucei* should reflect the further adaptation of this parasite to its parasitic niches: the digestive tract of the tsetse fly, which feeds only on blood, and the glucose-rich bloodstream of the mammalian host. Within this scenario, the occurrence of glucose as the major carbohydrate made the need for metabolism of other sugars redundant and the genes facilitating the metabolism of sugars other than glucose were subsequently lost (Berriman et al., 2005).

Lateral gene transfer also appears to extend towards the maintenance of the mitochondrial genome (kinetoplast) on which several critical components of the respiratory chain and ATP synthase are encoded. The original DNA polymerase gamma, found in the mitochondria of all other eukaryotes, is not present in trypanosomatids, but is replaced by not less than six DNA polymerases (Klingbeil et al., 2002). Several of these polymerases, POL1B, POL1C and POL1D, are closely related to a DNA

polymerase present in bacteriophages from the caudovirales group. (Opperdoes & Michels, 2006). Mitochondrial DNA ligase $k\alpha$ and $k\beta$ genes (Downey et al., 2005; Liu et al., 2005) are also closely related to the corresponding genes of the caudovirales group and of African swine fever virus. Both types of virus are transmitted by arthropods, as are the trypanosomatids themselves. Thus, it is reasonable to postulate lateral transfer of genes from one or more of these viruses to a predecessor of the present-day trypanosomatids may very well have occurred inside the midgut of an arthropod host. Following the acquisition of DNA-polymerase and DNA-ligase genes, gene duplication events would have resulted in the formation of the *POLIB*, *POLIC* and *POLID* and ligase $k-\alpha$ and $k-\beta$ gene families present in extant trypanosomatids. Such events could have been a driving force in the evolution of the complex catenated DNA structure which is commonly referred to as kDNA (Opperdoes & Michels, 2006).

Plausible drug targets

Despite the many problems associated with drug development for tropical diseases, a key translational application of the *Trypanosoma* genome sequencing projects would be the development of effective, safe medicines to treat Chagas disease and sleeping sickness. Obvious drug targets would be pathways that are absent from the host or reactions catalysed by enzymes with structural properties that are distinct from host enzymes. In this regard, the characterisation of numerous enzymes with plant-like traits or that are present as a consequence of lateral gene transfer events from bacteria provide attractive targets for drug development. Glycolysis, which is a critical pathway in both mammals and the medically relevant *Trypanosoma* species, provides suitable drug targets by virtue of the distinctive structural properties exhibited by many of the *Trypanosoma* glycolytic enzymes. A good example might be phosphoglycerate mutase. All vertebrates have phosphoglycerate mutases that are dependent on glycerate-bisphosphate as co-factor, but the trypanosome mutase is a plant-like glycerate-bisphosphate-independent isofunctional enzyme (Chevalier et al., 2000). Even here, however, the trypanosomatid genome sequences provide new surprises since it now transpires that all trypanosomatids in fact contain three distinct putative phosphoglycerate mutase isoforms: the plant-like cofactor-independent isoform that was originally characterised and two cofactor-dependent mutases, of which one is of eubacterial ancestry and the other of archaeal ancestry. No information is available as to whether expression of the latter two genes is functionally significant in specific life-cycle stages in *T. brucei* and *T. cruzi*. Yet, with such precedents in mind it is perhaps important to try and move quickly on from the genetic validation of new drug targets and towards the chemical validation of candidates. A good example is the chemical validation of GPI-anchor biosynthesis as a drug target against African trypanosomes (Smith et al., 2004). Many of the drugs presently in use against Chagas disease and sleeping sickness interfere either with the synthesis of trypanothione (such as eflornithine), or increase oxidant stress (nifurtimox), or react directly with this cofactor (melarsen oxide). Thus, it is likely that further characterization of enzymes involved in oxidative stress protection will continue to be attractive targets for drug development. Novel drug targets might also include the novel pathway of myristic acid biosynthesis in bloodstream *T. brucei*.

Closing summary

Different aspects of trypanosome metabolism have been the subject of numerous biochemical investigations during the last five decades, but the release of complete genome sequences for *T. brucei* and *T. cruzi* has still provided us with many surprises. We have focused our discussion upon the metabolic network that is organized across cytoplasm, mitochondrion and glycosomes, rather than the transporters and permeases which are located on the plasma membrane. Some types of permease often provide the delivery routes for anti-parasitic drugs, and changes in their substrate specificity or expression levels can result in drug resistance. The organization of some gene families that encode permeases could also have been used to promote the concepts of gene dosage as a way to increase gene expression [e.g. kinetoplastid glucose transporters (Tetaud et al. 1997)], divergence of isoform function following gene duplication events [e.g. kinetoplastid glucose transporters (Tetaud et al., 1997) and a nucleoside/nucleobase transporter family in *T. brucei* (Sanchez et al., 2002)], or to invoke further plant-like traits and horizontal gene transfer (Klaus et al., 2005), too. We have also described the metabolic insight that can be obtained when genome sequences are combined with other modern approaches for studying biology [e.g. proteomics (Atwood et al., 2005)]. However, we are a long way from being able to conclude the relative contributions that many individual pathways within the elaborate metabolic maps of *T. brucei* and *T. cruzi* (**Figures 1-3**) provide towards overall parasite fitness during natural transmission cycles between host and vector. Such comparative biochemistry is interesting in its own right, but is also likely to have relevance in the characterization of novel drug targets.

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Legends to figures

Figure 1. The pathways of core metabolism in *Trypanosoma*. Boxed metabolites are nutrients (in grey) or end-products (in black) of metabolism. Thick arrows represent major metabolite fluxes. Enzymes: 1, hexokinase; 2, phosphoglucose isomerase; 3, phosphofructokinase; 4, fructosebisphosphate aldolase; 5, triosephosphate isomerase; 6, glyceraldehyde-3-phosphate dehydrogenase; 7a, glycosomal phosphoglycerate kinase (bloodstream-form specific); 7b, cytosolic phosphoglycerate kinase (procyclic specific); 8, glycerol-3-phosphate dehydrogenase; 9 glycerol kinase; 10, adenylate kinase; 11, glycerol-3-phosphate oxidase (only in *T. brucei*); 12, phosphoglycerate mutase; 13, 2-enolase; 14, pyruvate kinase; 15, phosphoenolpyruvate carboxykinase; 16, malate dehydrogenase; 17, fumarate hydratase; 18, NADH-dependent fumarate reductase; 19, malic enzyme; 20, alanine aminotransferase; 21, glucosamine-6-phosphate deaminase; 22, pyruvate phosphate dikinase; 23 citrate synthase; 24, alpha-ketoglutarate dehydrogenase; 25, succinyl-CoA ligase; 26, succinate dehydrogenase; 27, acetate: succinate CoA transferase; 28, pyruvate dehydrogenase; 29, citrate lyase; 30, acetyl-CoA synthetase; 31, proline oxidation pathway; 32, histidine degradation pathway (only in *T. cruzi*); 33, threonine degradation pathway (only in *T. cruzi*); 34, threonine oxidation pathway (only in *T. brucei*).

Figure 2. The enzymes involved in the hexose-monophosphate pathway, purine salvage and pyrimidine biosynthesis which are present in glycosomes. Enzymes: 1, glucose-6-phosphate dehydrogenase; 2, 6-phosphogluconate dehydrogenase; 3, ribulose-5-phosphate 3-epimerase; 4, ribulose-5-phosphate isomerase; 5, transketolase; 6, transaldolase; 7, transketolase; 8, ribulokinase; 9, ribokinase; 10, xylulokinase; 11, AMP deaminase; 12, inosine-5'-monophosphate dehydrogenase; 13, xanthine phosphoribosyltransferase; 14, hypoxanthine-guanine phosphoribosyltransferase; 15, phosphoribosylpyrophosphate synthetase; 16 orotate phosphoribosyltransferase; 17, orotidine-5'-phosphate decarboxylase; 18, pyruvate:phosphate dikinase; 19, purine nucleosidase (inosine-uridine nucleoside hydrolase); 20, sedoheptulose-1,7-bisphosphatase; 21, fructose bisphosphate aldolase; 22, triose phosphate isomerase; 23, adenylate kinase.

Figure 3. Amino-acid metabolism and some associated pathways. Pathways indicated in black have been identified in at least one of the two organisms. Dotted line represent reactions catalysed by enzymes only found in *T. cruzi*, Dashed lines represent reactions catalysed by enzymes only found in *T. brucei*. Pathways in grey are absent from both, but present in most other eukaryotes. Question marks represent enzyme catalysed steps for which no unambiguous gene identification could be made. Abbreviations used are: AcAc, acetoacetate; AdoMet, adenosylmethionine; B, biopterin; qH₂B, quinonoid form of dihydrobiopterin; H₂F, dihydrofolate; H₄F, tetrahydrofolate; HMG-CoA, hydroxymethylglutaryl coenzyme A; T[SH]₂, reduced trypanothione; T[S]₂, oxidised

trypanothione; MeG, methylglyoxal; GSH, glutathione; RSH, reduced thiol; RSSR, oxidised thiol.

Table 1A. Enzymes of carbohydrate metabolism

Enzyme name	EC number	T. brucei accession	T. cruzi accession	Copypnumber
6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase	2.7.1.105/3.	Tb05.6E7.310	Tc00.1047053511649.150	■
6-phosphogluconate dehydrogenase	1.1.1.44	Tb09.211.3180	Tc00.1047053510663.70	■
6-phosphogluconolactonase	1.1.1.44	Tb11.02.4200	Tc00.1047053503945.40	■
alternative oxidase	1.1.3.21	Tb10.6k15.3640	Tc00.1047053504147.180	■
fructose-1,6-bisphosphatase	3.1.3.11	Tb09.211.0540	Tc00.1047053506649.70	■
fructose-1,6-bisphosphate aldolase	4.1.2.13	Tb11.23.0003	Tc00.1047053504163.50	■
fucose kinase	2.7.1.52	absent	Tc00.1047053508377.9	■
fumarate hydratase	4.2.1.2	Tb03.2617.860	Tc00.1047053507257.60	■
fumarate hydratase (mitochondrial)	4.2.1.2	Tb11.02.2700	Tc00.1047053507669.10	■
galactokinase	2.7.1.6	absent	Tc00.1047053507001.110	■
glucokinase	2.7.1.2	absent	Tc00.1047053510187.100	■
glucose-6-phosphate 1-dehydrogenase	1.1.1.49	Tb10.70.5200	Tc00.1047053509287.50	■
glucose-6-phosphate isomerase	5.3.1.9	Tb927.1.3830	Tc00.1047053510889.221	■
glucose transporter THT1		Tb10.6k15.2030	Tc00.1047053506355.10	■
glyceraldehyde 3-phosphate dehydrogenase, glycosomal	1.2.1.12	Tb06.2669.1030	Tc00.1047053509065.70	■
glyceraldehyde 3-phosphate dehydrogenase, cytosolic	1.2.1.12	Tb10.6k15.3850	Tc00.1047053511235.20	■
glycerol kinase, glycosomal	2.7.1.30	Tb09.211.3550	Tc00.1047053510661.60	■
glycerol-3-phosphate dehydrogenase, FAD dependent mitochondrial	1.1.99.5	Tb11.02.5280	Tc00.1047053511151.90	■
glycerol-3-phosphate dehydrogenase (FAD-dependent), putative	1.1.99.5	Tb927.1.1130	Tc00.1047053511423.70	■
glycerol-3-phosphate dehydrogenase [NAD], glycosomal	1.1.1.8	Tb08.2811.740	absent	■
hexokinase	2.7.1.1	Tb10.70.5820	Tc00.1047053510121.20	■
L-gulonolactone oxidase	1.1.3.8	Tb05.26K5.770	Tc00.1047053509179.100	■
malate dehydrogenase-like	1.1.1.37	Tb10.70.5120	Tc00.1047053507883.100	■
malate dehydrogenase (mitochondrial)	1.1.1.37	Tb10.70.5110	Tc00.1047053506195.110	■
malate dehydrogenase (glycosomal)	1.1.1.37	Tb10.61.0980	Tc00.1047053511293.69	■
malate dehydrogenase (cytosolic)/AHADH	1.1.1.37	Tb11.01.3040	Tc00.1047053506937.10	■
NADH-dependent fumarate reductase, FRDg	1.3.1.6	Tb05.28F8.460	Tc00.1047053510215.10	■
NADH-dependent fumarate reductase (FRDm1)	1.3.1.6	Tb10.70.3720	Tc00.1047053508535.10	■
phosphoenolpyruvate carboxykinase	4.1.1.49	Tb927.2.4210	Tc00.1047053508441.20	■
phosphofructokinase	2.7.1.11	Tb03.3K10.320	Tc00.1047053508153.340	■
phosphoglucosmutase	5.4.2.2	absent?	Tc00.1047053511911.130	■
phosphoglycerate kinase	2.7.2.3	Tb927.1.700	Tc00.1047053506835.70	■
phosphoglycerate mutase cofactor dependent-like	5.4.2.1	Tb05.6E7.310	Tc00.1047053511649.150	■
phosphomannomutase	3.1.3.11	absent	Tc00.1047053508257.70	■
phosphomannomutase, putative	3.1.3.11	Tb10.70.0370	Tc00.1047053510187.480	■
phosphomannose isomerase	5.3.1.8	Tb11.01.6410	Tc00.1047053511717.90	■
pyruvate carrier, mitochondrial possible		Tb11.01.5950	Tc00.1047053511725.140	■
pyruvate carrier, plasma membrane, possible		Tb05.29K2.510	Tc00.1047053508397.70	■
pyruvate, phosphate dikinase	2.7.9.1	Tb11.02.4150	Tc00.1047053506297.190	■
ribokinase	2.7.1.15	Tb11.03.0090	Tc00.1047053506925.480	■
ribose 5-phosphate isomerase	5.3.1.6	Tb11.01.0700	Tc00.1047053508601.119	■
ribulokinase	2.7.1.16	absent	Tc00.1047053508625.150	■
sedoheptulose-1,7-bisphosphatase	3.1.3.37	Tb927.2.5800	Tc00.1047053504117.20	■
transaldolase, probable	2.2.1.2	Tb08.26E13.220	Tc00.1047053503477.20	■
transketolase	2.2.1.1	Tb08.11J15.550	Tc00.1047053511067.30	■
UDP-galactose 4-epimerase	5.1.3.2	Tb11.02.0330	Tc00.1047053506811.190	■
UDP-glucose pyrophosphorylase	2.7.7.9	Tb10.389.0330	Tc00.1047053506359.60	■

fatty acid elongase, putative	2.3.1.119	Tb07.5F10.380	Tc00.1047053511245.140	■	■
fatty acid elongase, putative	2.3.1.119	Tb07.5F10.400	Tc00.1047053511245.150	■	■
enoyl-CoA hydratase (peroxisomal)	4.2.1.17	Tb03.48K5.550	Tc00.1047053511277.210	■	■
fatty acyl CoA synthetase	6.2.1.3	Tb10.70.4200	Tc00.1047053510943.33	■	■
long-chain-fatty-acid-coA ligase protein	6.2.1.3	Tb11.02.5520	Tc00.1047053511693.100	■	■
malic enzyme-NADP (cytosolic)	1.1.1.40	Tb11.02.3120	Tc00.1047053505183.30	■	■

Table 1E. Enzymes of phospholipid metabolism

Enzyme name	EC number	T. brucei accession	T. cruzi accession	Copypnumber
1-acyl-sn-glycerol-3-phosphate acyltransferase-like protein	2.3.1.23	Tb11.01.6800	Tc00.1047053509157.60	■
alkyl-dihydroxyacetonephosphate synthase	2.5.1.26	Tb06.28P18.170	Tb06.28P18.170	■
cdp-diacylglycerol synthetase-like protein	2.7.7.41	Tb07.8P12.300	Tc00.1047053511237.40	■
cholinephosphate cytidylyltransferase-like protein	2.7.7.15	Tb10.389.0730	Tc00.1047053509805.220	■
diacylglycerol cholinephosphotransferase	2.7.8.2	Tb10.6K15.1570	Tc00.1047053509791.150	■
diacylglycerol kinase-like protein	2.7.1.107	absent	Tc00.1047053507257.80	■
dihydroxyacetone phosphate acyltransferase	2.3.1.42	Tb04.2H8.550	Tc00.1047053504055.40	■
ethanolamine-phosphate cytidylyltransferase-like protein	2.7.7.14	Tb11.01.5730	Tc00.1047053511727.120	■
glycerophosphoryl diester phosphodiesterase, probable	3.1.4.46	Tb11.01.0580	Tc00.1047053511071.100	■
glycosylphosphatidylinositol-specific phospholipase C	4.6.1.14	Tb927.2.6000	Tc00.1047053506617.70	■
lysophospholipase	3.1.1.5	Tb08.11J15.160	Tc00.1047053511907.50	■
myo-inositol monophosphatase, putative	3.1.3.25	Tb05.26K5.720	Tc00.1047053507047.120	■
phosphatidic acid phosphatase protein-like	3.1.3.4	Tb08.25E9.660	Tc00.1047053503809.110	■
Phosphatidylinositol-specific phospholipase-like protein	3.1.4.11	Tb11.02.3780	Tc00.1047053504149.160	■
phosphatidylinositol synthase-like protein	2.7.8.11	Tb09.160.0530	Tc00.1047053510349.50	■
Phosphatidylserine decarboxylase proenzyme-like protein	4.1.1.65	Tb09.211.1610	absent	■
phosphatidylserine synthase-like	2.7.8.8	Tb07.28B13.50	Tc00.1047053509937.30	■
phosphoenolpyruvate mutase	5.4.2.9	absent	Tc00.1047053511589.140	■
phosphoinositide-specific phospholipase C	3.1.4.11	Tb11.02.3780	Tc00.1047053504149.160	■
phospholipase A1, putative	3.1.1.32	Tb927.1.4830	absent	■
phospholipase A2-like protein	3.1.1.4	Tb09.211.3650	Tc00.1047053510743.60	■

Table 1F. Enzymes of oxidant stress protection

Enzyme name	EC number	T. brucei accession	T. cruzi accession	Copypnumber
ascorbate-dependent peroxidase	1.11.1.11	absent	Tc00.1047053506193.60	■
GPX1 trypanothione/tryparedoxin dependent peroxidase	1.11.1.9	Tb07.27M11.270	Tc00.1047053503899.110	■
iron/ascorbate oxidoreductase family protein, (glycosomal)	1.15.11	Tb05.25N21.30	Tc00.1047053510141.10	■
iron superoxide dismutase (SODA, mitochondrial)	1.15.11	Tb05.27M3.490	Tc00.1047053509775.40	■
iron superoxide dismutase (SODC, mitochondrial)	1.15.11	Tb11.01.7480	Tc00.1047053506819.30	■
iron superoxide dismutase (SODB2 glycosomal)	1.15.11	Tb11.01.6660	Tc00.1047053511019.90	■
iron superoxide dismutase (SODB1 cytosolic)	1.15.11	Tb11.01.7550	Tc00.1047053511715.10	■
trypanothione reductase	1.8.1.12	Tb10.406.0520	Tc00.1047053503555.30	■
tryparedoxin peroxidase peroxiredoxin		Tb09.160.4250	Tc00.1047053507259.10	■
TRYP1 tryparedoxin		Tb03.28C22.740	Tc00.1047053509997.30	■
tryparedoxin, putative		Tb03.48K5.910	Tc00.1047053509997.20	■

Table 1G. Enzymes of purine and pyrimidine metabolism

Enzyme name	EC number	T. brucei accession	T. cruzi accession	Copypnumber
adenylosuccinate lyase	4.3.2.2	Tb09.160.5560	Tc00.1047053503855.30	■
AMP deaminase (glycosomal)	3.5.4.6	Tb11.02.1340	Tc00.1047053506733.20	■
aspartate carbamoyltransferase	2.1.3.2	Tb05.6E7.770	Tc00.1047053511923.110	■

Glycosome



