

Surprising variety in energy metabolism within Trypanosomatidae

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The metabolism of Trypanosomatidae differs significantly between distinct species and can even be completely different between various life-cycle stages of the same species. It has been proposed that differences in energy metabolism are related to differences in nutrient supply in the environments of the various trypanosomatids. However, the literature shows that availability of substrates does not dictate the type of energy metabolism of trypanosomatids, as *Trypanosoma theileri*, *Trypanosoma lewisi* and African trypanosomes all live in the bloodstream of their mammalian host, but have surprisingly large differences in metabolism. Furthermore, in trypanosomatids no obvious relationship exists between energy metabolism and phylogeny or mode of transmission. We provide an overview of the metabolic capacities in the energy metabolism of distinct trypanosomatids, and suggest that these can be divided into four different metabolic categories of increasing complexity.

Introduction to the energy metabolism of Trypanosomatidae

The family of Trypanosomatidae, of which all members studied so far are parasites, is part of the protozoan order Kinetoplastida. Many trypanosomatids are of clinical interest because they cause important diseases, such as Chagas disease, leishmaniasis and sleeping sickness in humans, and nagana in livestock. Trypanosomatids possess several striking biological characteristics, such as a kinetoplast containing the catenated circular network of mitochondrial DNA, and RNA editing, a process where mitochondrial transcripts are modified by insertions or deletions of uridines [1,2]. The energy metabolism of trypanosomatids is also special because: (i) a large part of glycolysis is performed in an organelle, the glycosome [3]; and (ii) the electron transport chains of many trypanosomatids use an alternative oxidase that is otherwise found only in some fungi and plants (Figure 1).

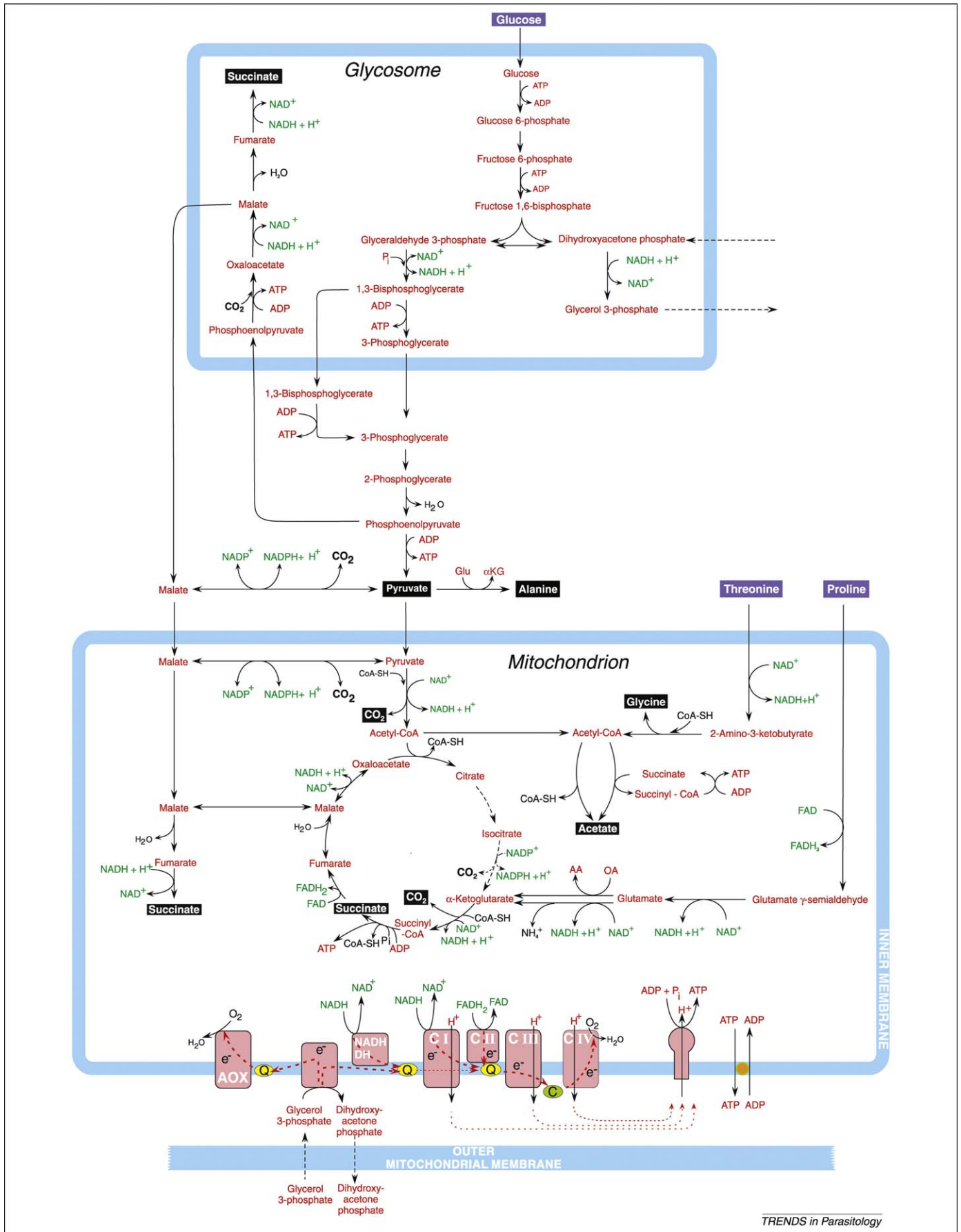
This review deals with variations in energy metabolism within the trypanosomatid family. Comparisons of the main aspects of energy metabolism in the trypanosomatid family are feasible, although the literature on this subject is biased as most studies have been performed on some selected species, such as *Trypanosoma brucei*, *T. cruzi* and *Leishmania* spp. Even within this restricted number of selected species, studies have mainly been focused on two life cycle stages of *T. brucei*: the bloodstream form from the

mammalian host, and the procyclic form, which lives in the tsetse midgut. *In-silico*-derived metabolic maps drawn from genome-sequencing projects show all theoretically possible pathways of the species in question. Such maps are of course valuable by themselves, as they provide information on the metabolic potential of various life cycle stages, but they give no clues on the relative importance of the fluxes through the various pathways. Discussion of the energy metabolism of trypanosomatids will be restricted in this review to the main catabolic pathways. For more details on all possible metabolic pathways the reader is referred to more extensive reviews on those subjects [4–11]. In this review, an overview of the metabolic capacities in trypanosomatid energy metabolism will be discussed and, to stimulate further discussion, an attempt is made to categorize these variations.

Habitats

Trypanosomatids are classified into several genera, and include monogenetic parasites of insects, as well as parasites that alternate in their life cycle between insects and vertebrates (or between insects and plants in case of *Phytomonas*). Accordingly, there are many different habitats in which trypanosomatids live. The monogenetic *Herpetomonas*, *Leptomonas* and *Crithidia* species spend their entire lifetime in the intestinal tract of insect hosts. The intestine is also the habitat of all trypanosomatids with two hosts when the parasite enters the insect vector: the *T. brucei* clade in tsetse flies, *T. cruzi* in triatome bugs, *Leishmania* spp. in sand flies, and *Phytomonas* in phytophagous insects. Trypanosomatids often have a complex development within the insect host, and migrate to environments other than the midgut, ending up for instance in the salivary glands. Metabolic aspects of insect stages other than those in the intestinal midgut are, however, hardly studied. Apart from *Phytomonas*, which infects plants, all known trypanosomatids with two hosts parasitize vertebrates as their second host. Trypanosomatids live in two completely different habitats within these vertebrate hosts: either in the bloodstream or intracellularly, depending on the species. For example, African salivarian trypanosomes, such as *T. brucei*, generally live in the bloodstream of mammals, ranging from cattle to humans. By contrast, amastigotes of *T. cruzi* live intracellularly in the cytoplasm of different cell types of their vertebrate host; whereas amastigotes of *Leishmania* live in a parasitophorous vacuole within macrophages of their mammalian host.

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Figure 1. Generalized schematic representation of the major metabolic pathways in the energy metabolism of trypanosomatids. Substrates are represented in blue boxes and end products in black boxes. Abbreviations: AA, amino acids; α-KG, α-ketoglutarate; AOX, alternative oxidase; c, cytochrome c; CI-IV, complexes I-IV of the electron-transport chain; OA, 2-oxoacids; Q, ubiquinone.

Substrates for energy metabolism in trypanosomatids

Studies on energy metabolism deal with the ways organisms produce ATP for their functioning and proliferation. Three different classes of substrates can, in principle, be used for the production of ATP: carbohydrates, fatty acids and amino acids. All eukaryotes, including trypanosomatids, can use carbohydrates if these are available. Many trypanosomatids live in carbohydrate-rich environments during their stay inside their non-insect host, vertebrates, or plants. For instance, *T. brucei*, *T. lewisi* and *T. theileri* live in the bloodstream of their vertebrate host where the glucostasis of the host guarantees a continuous supply of glucose. *Phytomonas* spp. live in specific carbohydrate-rich environments in plants, such as phloem and fruits, and excrete enzymes that enable them to utilize several plant-specific carbohydrates, such as cellulose, starch, sucrose and xylulose [12]. In addition, *L. major* is also adapted to obtain sugars, using an amylase, many sugar kinases and a sucrose-like protein for the digestion of plant starch and disaccharides, which suggests that these organisms are adapted to digest the various sugars present in the nectar upon which the sandfly feeds in addition to its bloodmeals [13].

Glucose, however, is not always abundant in the habitats of trypanosomatids. In insect intestines, a niche where all trypanosomatids spend at least part of their life cycle, carbohydrates are often sparse and parasites living there have to also use other substrates for their energy supply. African trypanosomes, *T. cruzi* and *Leishmania* spp. that inhabit insect intestines, and trypanosomatids that live inside mammalian cells, such as amastigote stages of *T. cruzi* and *Leishmania* spp., use the abundantly available amino acids (especially proline and glutamine) as substrates for energy metabolism via mitochondrial pathways [5,14–16].

Fatty acids are not known to be an important substrate used in the energy metabolism of trypanosomatids. Exceptions are probably the amastigotes of *Leishmania* and *T. cruzi* [10,17]. Studies on *Leishmania mexicana* amastigotes showed that this stage has increased metabolism of fatty acids and a reduced use of proline and glucose compared to promastigotes [18–20].

Metabolic pathways and end products

Glycolysis

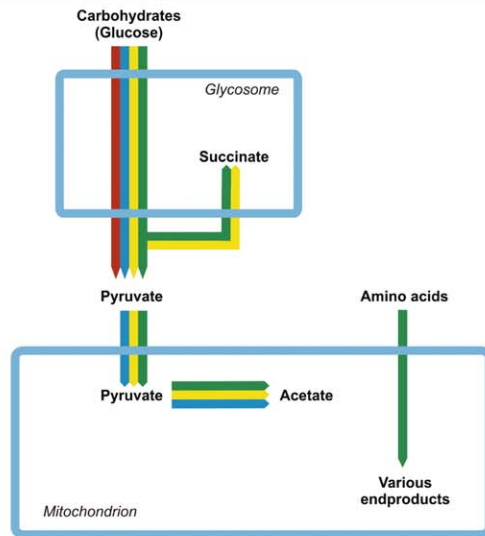
Glycolysis is a metabolic pathway that is present in all living eukaryotes, and trypanosomes are no exception. All stages of all trypanosomatids can degrade glucose, when present, to pyruvate using the classical Emden–Meyerhof pathway. However, in all Kinetoplastida studied so far, glycolysis differs significantly from that of all other eukaryotes, because a large part of the glycolytic pathway is sequestered within glycosomes, organelles of peroxisomal origin [3]. Furthermore, trypanosomes lack negative feedback regulation of early steps in glycolysis. These two peculiar properties of trypanosomatids are probably related. Computer simulations in combination with biochemical experiments demonstrated that the compartmentation (sequestration) of glycolysis in the glycosomes serves a regulatory function that compensates for the lack of classical regulation of this part of glycolysis [21].

Pyruvate

Pyruvate is the end product of glycolysis and glucose metabolism in long-slender bloodstream forms of African trypanosomatids (see Figures 1 and 2). Excretion of pyruvate as the main end product is highly unusual, even among trypanosomatids. In most eukaryotes, pyruvate is transported into the mitochondria for further oxidation and the accompanying ATP formation. If the use of the mitochondrial respiratory chain is prohibited, for instance by a lack of oxygen, pyruvate is usually not transported into the mitochondria, but acts as the final electron acceptor and is reduced in the cytosol, often to lactate by lactate dehydrogenase, in order to maintain redox balance. However, in several trypanosomatids, such as *T. brucei*, *T. cruzi* and *Leishmania* spp., lactate dehydrogenase is not present [22]. In the pyruvate producing bloodstream form, *T. brucei* redox balance is maintained using oxygen as the final electron acceptor via a glycerol-3-phosphate shuttle linkage to the plant-like alternative oxidase (Figure 1). It is striking that all stages of all trypanosomatids studied so far perform so-called aerobic fermentations (Box 1).

Acetate is a main end product of glucose degradation in all trypanosomatids, except for the long-slender bloodstream forms of the *T. brucei* family, which excrete pyruvate. In the other trypanosomatids, pyruvate is transported into the mitochondrion where it is isoxidatively decarboxylated to acetyl-CoA by the pyruvate dehydrogenase complex. Acetate is then produced predominantly by a two-enzyme cycle, in which acetate:succinate CoA-transferase (ASCT) transfers the CoA moiety of acetyl-CoA to succinate, yielding acetate and succinyl-CoA, which is subsequently converted back to succinate by succinyl-CoA synthetase, an enzyme that yields ATP and is also part of the Krebs cycle [23]. In trypanosomatids acetate can also be an end product of the mitochondrial degradation of amino acids (such as threonine) resulting in the formation of pyruvate, or acetyl-CoA directly [10,24,25]. Finally, as the end product of β -oxidation, acetyl-CoA, is not further metabolized via the Krebs cycle (see below), we propose that acetate is also the end product when fatty acids are degraded by amastigotes of *Leishmania* spp. and *T. cruzi*, but this needs further investigation.

Succinate is produced via (at least) three different pathways by trypanosomatids and is an end product of glucose and amino acid degradation (Figure 1). This production of succinate from glucose occurs in the glycosomes and in the mitochondrion by two different soluble NADH-dependent fumarate reductase isoenzymes [26,27]. In the glycosomes of procyclic *T. brucei*, and most likely in the glycosomes of other trypanosomatids as well, succinate is produced as an end product of glucose breakdown when glycolytically produced phosphoenolpyruvate is transferred from the cytosol into the glycosome. This phosphoenolpyruvate is then carboxylated by phosphoenolpyruvate carboxykinase, and converted via malate and fumarate into succinate, which is excreted (Figure 1). When malate resulting from glucose breakdown enters the mitochondrion, the same two final reactions occur, resulting again in the production of succinate. Succinate can also be the end product of the degradation of amino acids such as



Category ^a	1	2		3	4				
Life-cycle stage ^b	BSF	BSF	Plant stage	BSF	PC	BSF	TM, EM	AM, PM	CH
& trypanosomatid species	<i>T. brucei</i> <i>T. gambiense</i> <i>T. rhodesiense</i> <i>T. evansi</i>	<i>T. congolense</i>	<i>Phytomonas</i>	<i>T. theileri</i>	<i>T. brucei</i>	<i>T. lewisi</i>	<i>T. cruzi</i>	<i>Leishmania</i>	<i>Crithidia</i>
Carbohydrate degradation by glycolysis	+	+	+	+	+	+	+	+	+
Pyruvate as main end product	+	-	-	-	-	-	-	-	-
Acetate production	-	+	+	+	+	+	+	+	+
Succinate production	-	+/-	-	+	+	+	+	+	+
Amino acid catabolism	-	-	-	-	+	+	+	+	+
Cytochrome-containing respiratory chain	-	-	-	+	+	+	+	+	+
Plant-like alternative oxidase	+	+	+	-	+	^c	-	-	+
References	31, 36, 44, 45	41, 44, 46	12, 47, 48	39	25, 33	44,49	15, 50	10, 16, 18, 19, 51	52, 53

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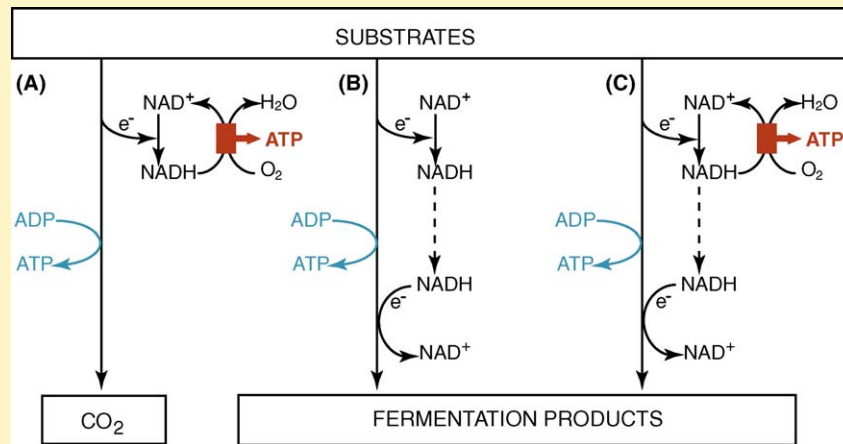
Figure 2. Overview of main metabolic capacities in the energy metabolism of distinct trypanosomatids.

^aEnergy metabolism of distinct trypanosomatids is classified in four categories of increasing complexity.

^bStages in mammalian host: AM, amastigote forms; BSF, long-slender bloodstream forms; TM, trypomastigote form; Stages in insect host: CH, choanomastigote; EM, epimastigote form; PC, procyclic form; PM, promastigote form.

^cnot yet reported.

Box 1. Types of energy metabolism



A) Aerobic energy metabolism is defined as the complete oxidation of substrates to carbon dioxide and water. The majority of ATP in aerobic energy metabolism is produced via the mitochondrial respiratory chain and oxidative phosphorylation. Oxygen acts as the final electron acceptor to re-oxidise the reduced coenzyme NADH.

B) Fermentation is defined as the degradation of substrates via processes that produce their own oxidants to balance production and re-oxidation of reduced coenzyme NADH. The fermentation of glucose to lactate is a well known example of such a process. In some cases fermentation is linked to an electron transport chain and oxidative phosphorylation (not shown in this figure). However, in that case the final electron acceptor is not oxygen, but for instance endogenously produced fumarate.

C) Aerobic fermentation is the term often used to describe the form of metabolism that is used by all trypanosomatids. In this process the substrates used in energy metabolism are not completely oxidised, but instead fermentation products such as acetate and succinate are excreted. However, in this case the electron transport chain uses oxygen as final electron acceptor. Depending on the composition of the electron-transport chain this process of oxidation of NADH can be accompanied by oxidative phosphorylation. It is remarkable that trypanosomatids on the one hand produce fermentation products under all conditions studied so far, but on the other hand need oxygen as final electron acceptor for optimal functioning and cannot really depend on true fermentative processes.

Substrate level phosphorylations are represented by blue arrows and oxidative phosphorylation is shown in red.

proline and glutamate [28]. In this case part of the Krebs cycle is used: α -ketoglutarate dehydrogenase and SCS catalyse the two final reactions of this succinate formation (Figure 1) [25]. In the absence of glucose most of the succinate formed from proline is further metabolized and is converted to alanine via pyruvate [29].

Krebs cycle activity was earlier thought to be present in trypanosomatids, at least in insect stages of some African trypanosomes [28,30–32]. However, for African trypanosomatids and *Leishmania* spp. this presumed flux through the Krebs cycle is only poorly supported by direct experimental evidence and was mainly based on the detected presence of enzyme activities. More recent studies revealed that at least in *T. brucei* and *Leishmania*, the Krebs cycle is not used for the complete oxidation of acetyl-CoA to carbon dioxide, although genes encoding all enzymes of this cycle are indeed present in the genome and expressed in those insect stages [22,25,33,34]. Instead, parts of the cycle are most likely used in anabolic pathways, such as gluconeogenesis and fatty acid formation, and also for the final steps in the degradation of amino acids (see above) [25]. It is unclear yet whether complete oxidation of substrates to carbon dioxide via the Krebs cycle occurs to a significant extent in other, not yet investigated stages of some trypanosomatids.

The electron transport chain composition is not the same in all trypanosomatids and varies from a very simple one in the mitochondrion of the long-slender bloodstream

form of *T. brucei* to a classical cytochrome-containing one in for instance *T. cruzi* and *Leishmania* spp. The mitochondrial functions of long-slender bloodstream form *T. brucei* are largely repressed, but the electron transport chain nevertheless also has an important function in this stage as it transfers electrons from the glycosomal oxidation of glucose to oxygen. This occurs in a rather unusual way, as this electron transport chain does not have cytochrome containing complexes, but instead contains a plant-like alternative oxidase [35]. Reducing equivalents (NADH) produced in the glycosomes are transferred to the mitochondrion via the glycerol-3-phosphate and dihydroxyacetone phosphate shuttle that is also present in mammals (Figure 1). This electron transport does not involve proton translocation and is therefore not linked to ATP production. Compared to the long-slender bloodstream forms of *T. brucei* spp., the mitochondria of procyclic stages contain a more classical respiratory chain, comprising trypanosomal homologues of the respiratory chain complexes (I, II, III and IV) that are present in mammalian species [7]. The mitochondria of insect stages of *T. brucei* have a branched electron transport chain, which contains four complexes that donate electrons to the ubiquinone pool, glycerol-3-phosphate dehydrogenase, an NADH:dehydrogenase, complex I, and complex II (Figure 1) [8]. The formed ubiquinol can then be reoxidized and transfers electrons to the complexes III and IV of the classical respiratory chain, or to the plant-like alternative oxidase (Figure 1).

As described above, the mitochondrial energy metabolism of procyclic *T. brucei* mainly depends on degradation of pyruvate to acetate, and of amino acids to succinate and/or acetate. Both catabolic pathways produce ATP by substrate level phosphorylation, because both pathways comprise succinyl-CoA to succinate conversion by SCS. However, a functional respiratory chain is also essential for these pathways, as both pathways also produce large amounts of NADH (Figure 1). This NADH is reoxidized by the respiratory chain and complete inhibition of the respiratory chain by anoxia or simultaneous inhibition of both the plant-like alternative oxidase and the complex IV of the classical respiratory chain, results in a rapid death of the cells [33] (Box 1).

Four categories of energy metabolism in trypanosomatids

The energy metabolism of trypanosomatids varies considerably, not only between species but also between distinct life cycle stages of the same species [5,16]. It has been suggested that the use of glycolysis solely, versus a more complex (mitochondrial) energy metabolism, correlates with the abundance of carbohydrate substrates for glycolysis in the natural environment of the distinct trypanosomatids [5,9,11,16]. This is not in agreement with the literature and we suggest that the observed variations in energy metabolism of trypanosomatids can be divided into four different categories with increasing complexity (Figure 2). In short: parasites in the first category are characterized by the production of pyruvate from glucose; in the second category the main end product of glucose breakdown is acetate instead of pyruvate; the third category is again more complex as these parasites also produce succinate from glucose; and finally the metabolism of the parasites in the fourth category is up to now the most complex one observed in trypanosomatids, as these also use amino acids in addition to glucose.

The first category comprises the long-slender bloodstream stages of the *T. brucei* family, *T. brucei brucei*, *T. b. gambiense*, *T. b. rhodesiense* and *T. evansi*. These bloodstream stages use exclusively glycolysis up to pyruvate to produce ATP. Significant amounts of acetate or succinate are not produced and these organisms do not consume amino acids. They do not utilize a classical cytochrome-containing respiratory chain and do not use oxidative phosphorylation for the production of ATP, which in the bloodstream stages of these species is solely produced by the substrate-level phosphorylations of glycolysis (Box 1). In the long-slender bloodstream stage these trypanosomatids rely solely on the plant-like alternative oxidase for the oxidation of the NADH that is produced during the formation of pyruvate. Compared to the metabolic capacities of the other trypanosomatids, the members of this category have the least complex, and therefore least flexible, energy metabolism. The relative unimportance of the mitochondrion for these bloodstream forms is reflected in the observation that mitochondrial DNA can be lost, as happened recently in *T. equiperdum* and *T. evansi*, which independently lost all or part of their kinetoplast DNA [36]. However, these organisms are then

locked in the bloodstream form, which probably indicates that expression of mitochondrial DNA is essential in certain stages in the insect host. Indispensable genes that are encoded on mitochondrial DNA could include those involved in the synthesis of functional ATP synthase or cytochrome-containing parts of the respiratory chain [36,37].

The second category contains the bloodstream stage of *T. congolense* and the plant stage *Phytomonas*. These trypanosomatids also rely solely on glucose for the production of ATP. However, they do not produce pyruvate, like the parasites in the first category, but form acetate as the main end product. Like the parasites in the first category, the members of the second category do not consume amino acids and do not utilize a classical cytochrome-containing respiratory chain or oxidative phosphorylation for the production of ATP. These trypanosomatids rely solely on the plant-like alternative oxidase to oxidize NADH, just like the parasites in the first category.

The third category contains, up to now, exclusively the bloodstream stage *T. theileri*. These trypanosomatids also do not use amino acids but solely use glucose for the production of ATP. Their main end products are acetate and succinate. As acetate production is significantly more than half the production of succinate, this implies that an additional electron acceptor is needed for the reoxidation of the NADH formed during acetate production [25,38]. However, in contrast to the parasites in the first and second category these trypanosomatids do not use a plant-like alternative oxidase to donate electrons to oxygen, but rather a classical cytochrome-containing respiratory chain, which is a strong indication for ATP production by oxidative phosphorylation. The metabolism of bloodstream *T. theileri* (third category) in fact closely resembles the metabolism of short-stumpy bloodstream *T. brucei*, which produces acetate and has metabolic capacities in between those of the long-slender bloodstream forms (first category) and the procyclic insect form (fourth category) [34,39–42].

The fourth category contains the trypanosomatids with the most complex metabolic capacities. In this category are the bloodstream form *T. lewisi*, procyclic *T. brucei* spp., and all investigated stages in the mammalian and insect hosts of *T. cruzi* and *Leishmania* spp. In contrast to the parasites in the first, second and third category these trypanosomatids are not solely dependent on glucose. Trypanosomatids in the fourth category all have a more complex mitochondrial metabolism and can degrade amino acids in addition to glucose. They all use a cytochrome-containing respiratory chain and oxidative phosphorylation for the generation of ATP. Whereas *T. cruzi* and *Leishmania* spp. are solely dependent on a cytochrome-containing respiratory chain, the procyclic stage of *T. brucei* also uses the plant-like alternative oxidase. Although the catabolic capacities of the trypanosomatids in this category are very similar, differences do exist in their biosynthetic complexity. From analysis of their genomes it was concluded that *Leishmania* spp. have retained more anabolic capacities than *T. cruzi*, which in turn is more versatile than *T. brucei* [22].

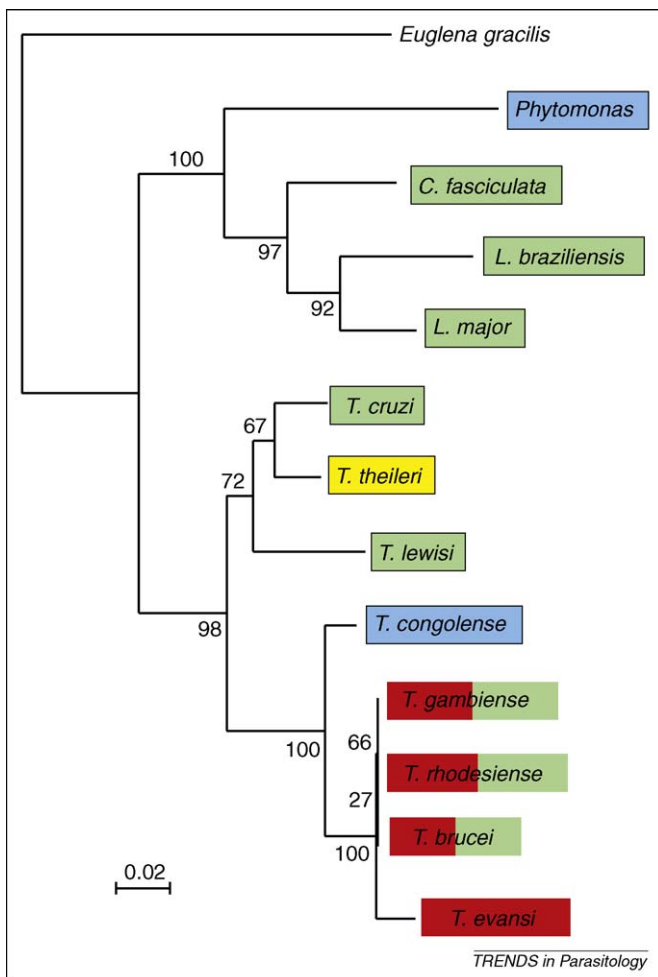


Figure 3. Phylogenetic tree of Trypanosomatidae. Neighbour-joining phylogenetic analysis of partial glyceraldehyde 3-phosphate dehydrogenase (GAPDH) sequences of Trypanosomatidae. Amino-acid sequences of equal length were aligned using ClustalX and phylogenetic trees were prepared using *Euglena gracilis* as an outgroup. Bootstrap values for 100 independent random order analyses are shown and the scale bar corresponds to 0.02 substitutions per site. Coloured boxes indicate the category of energy metabolism of the various trypanosomatids according to Figure 2. Sequences for the GAPDH genes of the shown species were obtained from Genbank (<http://www.ncbi.nlm.nih.gov/genbank/index.html>).

Correlations between metabolic category and habitat, mode of transmission, or phylogeny?

From Figure 2 it can be concluded, first, that only trypanosomatids living in carbohydrate-rich habitats, such as the mammalian bloodstream or plant tissues, rely solely on carbohydrates in their energy metabolism and do not use a cytochrome-containing respiratory chain or an elaborate mitochondrial metabolism. These parasites belong to the first, second and third category. However, not all trypanosomatids living in a carbohydrate-rich habitat rely solely on carbohydrates in their energy metabolism. This can be concluded from the metabolism of *T. lewisi*, which although it lives in the bloodstream of its mammalian host, uses amino acids in addition to glucose as substrates and further has a cytochrome-containing respiratory chain, thereby belonging to the fourth category. Apparently, the availability of different substrates, carbohydrates and/or amino acids, does not necessarily dictate the nature of the metabolic pathways employed.

Furthermore, Figure 2 shows that all trypanosomatids that consume amino acids in their energy metabolism possess a classical cytochrome-containing respiratory chain. These trypanosomatids form the fourth category. However, not all trypanosomatids with a classical cytochrome-containing respiratory chain use amino acids in their energy metabolism, as *T. theileri* uses a cytochrome-containing respiratory chain, but does not consume proline or glutamine, amino acids that are used by all other trypanosomatids known to utilize amino acids for the production of ATP [39].

In addition, there is not an obvious relationship in trypanosomatids between phylogeny and energy metabolism. Within the phylogenetic tree of the trypanosomatids that are subject of this review, three groups can be distinguished: i) the African salivarian trypanosomes; ii) the stercoraria *T. cruzi*, *T. theileri* and *T. Lewesi*; and iii) the group containing *Phytomonas*, *C. fasciculata* and *Leishmania* spp. (Figure 3). First of all, none of these three phylogenetically distinguishable groups comprises only trypanosomatids with a metabolism of one particular category. Second, trypanosomatids with the same category of metabolism can be found in distinct phylogenetic groups. For instance, plant-stage *Phytomonas* species and the bloodstream form *T. congolense* share many characteristics in energy metabolism (Figure 2), but are not closely related (Figure 3).

Apparently, there seems to be also no correlation between the way trypanosomatids are transmitted and their energy metabolism. Trypanosomes of the Stercoraria section that are transmitted via faeces are found in the third and fourth category, but this fourth category also contains the insect stages of the *T. brucei* family, which is part of the

Box 2. Different types of metabolic adaptations

Adaptations can be seen as modifications of the characteristics of organisms that facilitate an enhanced ability to survive and reproduce in a particular environment. These changes in characteristics can be phenotypic as well as genotypic. Within the trypanosomatids, both types of adaptations are present.

(i) Stage-specific gene expression during differentiation

During their life cycles, most trypanosomatids encounter various environments that differ significantly in the availability of different nutrients. Therefore, trypanosomatids often show developmental differences in energy metabolism during their life cycle, as they have to adjust their metabolism to the substrates available in the new niche. They then have to re-route their metabolic fluxes and this is performed by the expression of other genes. All trypanosomatids have a large repertoire of possible metabolic pathways, which allows immediate flexibility to adjust the fluxes through these metabolic pathways after possible variations in substrate availability in the environment. However, the repertoire is different in each of the four categories and determines the metabolic flexibility of the parasite in question.

(ii) Evolutionary loss or gain of certain genes in various trypanosomatids

During evolution, a continuous loss and gain of genes can occur. In trypanosomatids, the residence in various environments in the different hosts resulted in a differential loss (and gain) of specific genes depending on the availability of various compounds in the specific niches inside their hosts. As a result, genomic differences in energy metabolism are now observed between the different trypanosomatids.

Salivaria section and are transmitted via blood sucking. Trypanosomes of the Salivaria section are found in the first, second and fourth category. As the first category contains the long-slender bloodstream stages of exclusively the *T. brucei* family, this category only contains trypanosomes of the section Salivaria. However, this first category contains *T. brucei* spp. transmitted via blood sucking, but also *T. evansi* that is transmitted mechanically during bites.

Concluding remarks

How did this variety in adaptations and metabolic capacities arise? (See also Box 2) Like all parasites, after the transition from a free-living to a parasitic way of life, trypanosomatids could discard many metabolic capacities (i.e. those metabolic intermediates that could from now be obtained from the host). These adaptations to a new environment occurred gradually; and variations in the extent to which disposal of the metabolic capacities of the parasite occurred might explain why certain trypanosomatids have different metabolic capacities than others, although they live in the same environment. This is the case, for instance, in bloodstream forms of *T. brucei*, *T. theileri* and *T. lewisi*, which all live in a stable glucose-rich environment (Figure 2). Although more common in the bacterial world, it is surprising that closely-related eukaryotes living in the same habitat, show such a variation in energy metabolism. It is possible that the metabolic strategy of *T. theileri* and *T. lewisi* will ultimately transform into the same strategy as that of the least complex trypanosomatid, *T. brucei*, because evolution is a never-ending story, as was expressed exactly 150 years ago [43].

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