Evolution, dynamics and specialized functions of glycosomes in metabolism and development of trypanosomatids

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Abstract

Kinetoplastea such as trypanosomatid parasites contain specialized peroxisomes that uniquely contain enzymes of the glycolytic pathway and other parts of intermediary metabolism and hence are called glycosomes. Their specific enzyme content can vary strongly, quantitatively and qualitatively, between different species and during the parasites’ life cycle. The correct sequestering of enzymes has great importance for the regulation of the trypanosomatids’ metabolism and can, dependent on environmental conditions, even be essential. Glycosomes also play a pivotal role in life-cycle regulation of Trypanosoma brucei, as the translocation of a protein phosphatase from the cytosol forms part of a crucial developmental control switch. Many glycosomal proteins are differentially phosphorylated in different life-cycle stages, possibly indicative for unique forms of activity regulation, whereas many kinetic activity regulation mechanisms common for glycolytic enzymes are absent in these organisms. Glycosome turnover occurs by autophagic degradation of redundant organelles and assembly of new ones. This may provide the trypanosomatids with a manner to rapidly and efficiently adapt their metabolism to the sudden, major nutritional changes often encountered during the life cycle. This could also have helped facilitating successful adaptation of kinetoplastids, at multiple occasions during evolution, to their parasitic life style.

Introduction

Trypanosomatids are organisms that belong to the Kinetoplastea, a large group of flagellated free-living and parasitic protists, which are characterized by a number of unique features. Most notable is the possession of a kinetoplast, a special part of these organisms’ single mitochondrion containing a complex DNA structure, called kDNA. Another characteristic is the presence of glycosomes, unusual peroxisomes that harbour the major part of the glycolytic pathway, hence their name. All known trypanosomatid species are parasitic, infecting a large variety of organisms such as mammals – including human – reptiles, insects and even plants. The human-infective parasites, which are transmitted by insects, are responsible for potentially deadly diseases.

These parasites have a complicated life cycle; they are transmitted between humans and other mammals by insects. Trypanosoma brucei, that causes sleeping sickness in people living in sub-Saharan Africa, is introduced in the human bloodstream by the bite of an infected tsetse fly when taking a bloodmeal. The trypanosomes multiply as so-called long-slender bloodstream forms (BSF) in the circulatory system of the host where they subsequently differentiate into non-replicating short-stumpy forms that are pre-adapted to life in a new fly. In the fly’s midgut, the stumpy forms develop into proliferating procyclic forms (PCF) that, while undergoing several consecutive morphological changes, migrate to the salivary glands. Trypanosoma cruzi, responsible for Chagas disease in Latin-America, is transmitted between humans and animals by a blood-sucking reduviid bug, which deposits its infective faeces on the skin. After entry through skin wounds and mucous tissues, the insect-specific epimastigote form of T. cruzi transforms into the trypomastigote bloodstream forms that invade various host cells. They multiply as non-motile amastigotes in the cytosol of these cells and differentiate into new trypomastigotes that are released in the blood to infect other cells or to be ingested by a new bug in which they transform into epimastigotes. Different species of the genus Leishmania are responsible for a variety of cutaneous and visceral diseases worldwide in tropical and subtropical regions. The parasites are transmitted between human beings and animal reservoirs by the bite of a phlebotomine sandfly, where they are present in the proboscis as promastigote forms. Inside the mammalian host, promastigotes are phagocytized by macrophages. Within phagolysosomes of macrophages they transform into
amastigotes and multiply. A next sandfly taking a bloodmeal ingests and disintegrates the infected macrophages and the freed parasites transform within the midgut of the insect into promastigotes that migrate to the proboscis.

The different life-cycle stages of these parasites rewire their metabolism in order to persist and grow in the different hosts or host compartments. The metabolic changes largely reflect the availability of nutrients in the specific environment. For example, inside the mammalian bloodstream glucose is abundantly available and BSF T. brucei only uses – aerobic -glycolysis to generate free energy. The BSF trypanosomes can only for short periods live in anoxic conditions. In contrast, in the insect midgut, glucose is only available briefly after the fly has taken a bloodmeal and PCF T. brucei has expanded its metabolic repertoire in order to also metabolize amino acids like proline and threonine in its mitochondrion. In the course of mammalian infections, the stumpy forms that accumulate already partly induce expression of PCF-specific metabolic pathways, thereby allowing efficient progression through the life-cycle. Similarly, T. cruzi and Leishmania species adapt their metabolic network for optimally using the nutrients found when being shuttled between the very different environments of the insect alimentary tract and the cytosol of human cells and the phagolysosomes of macrophages, respectively.

Trypanosomatids contain 10 – 100 glycosomes per cell, dependent on the species and the life-cycle stage. For example, T. brucei, when living in the bloodstream of its mammalian host, contains about 65 glycosomes, with an average diameter of 0.27 µm. T. cruzi has approximately 50 glycosomes in its different life-cycle stages, and a similar number has been reported for cultured promastigotes of Leishmania spp. However, 5 - 10 times less glycosomes per cell are found in the much smaller intracellular amastigote form of Leishmania.

**Glycososomal metabolism**

Trypanosomatids can metabolise various carbon sources, but the exact metabolic set-up is dependent on the life-cycle stage of the parasites and largely reflects the substrate availability in the environment within the different hosts. In all developmental stages, glucose can be broken down to pyruvate via the glycolytic pathway (Fig. 1A). The reactions converting glucose to 3-phosphoglycerate occur inside glycosomes while further conversion to pyruvate takes place in the cytosol. Depending on the life-cycle stage and specific environmental conditions, pyruvate is excreted or further metabolised in the cytosol or mitochondrion (reviewed in [1-3]).

Also other enzymes, involved in either catabolic or anabolic processes, may be present in the organelles, as discussed elsewhere [4-7]. Most of these additional processes are coupled to the glycosomal ATP/ADP and NAD(P)/NAD(P)H pools. An example is the pentose phosphate pathway (PPP), a side-branch of glycolysis that produces NADPH and ribose 5-phosphate. The PPP enzymes are dually located in glycosomes and cytosol, the distribution being species and life-cycle stage dependent [3,7].

Within the glycosomes of BSF T. brucei, there is an ATP/ADP balance for glycolysis (Fig. 1B): two glycosomal ATP consuming reactions reside in the upper part, followed by two ATP producing reactions in the lower part. An NAD+/NADH balance is also maintained within the organelles. The NADH formed in the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) reaction is reoxidized by reduction of dihydroxyacetone phosphate (DHAP) to glycerol 3-phosphate (G3P). Under aerobic conditions, G3P is shuttled to a short respiratory system
with a so-called alternative oxidase (AOX) in the mitochondrial membrane that is not coupled to oxidative phosphorylation. The produced DHAP will re-enter the glycosomes and is further metabolized in the pyruvate branch. Without oxygen, the G3P is converted into glycerol plus ATP by a glycerol kinase, thermodynamically enabled at a low glycosomal ATP/ADP ratio. Whereas under aerobic conditions trypanosomes hardly produce glycerol from glucose and regenerate all ATP in the pyruvate branch, under anaerobiosis equimolar amounts of glycerol and pyruvate are made, and one ATP molecule is regenerated in each branch. Different species and life-cycle stages may use alternative side-branches to glycolysis in order to regenerate NAD⁺ and maintain the redox balance inside glycosomes. Cultured PCF of T. brucei have an extended glycosomal metabolism: phosphoenolpyruvate can re-enter the organelles and be converted to succinate or pyruvate to regenerate glycosomal NAD⁺ and ATP (Fig. 1C) [8].

T. cruzi and Leishmania spp. live extracellularly in their insect vectors but reside and proliferate predominantly intracellularly, as so-called amastigote forms, in their mammalian hosts. The metabolic capabilities of these parasites appear to be largely similar to those of PCF T. brucei, but fatty acids seem a main source of energy in the intracellular stages. Fatty-acid β-oxidation enzymes localise not only inside the mitochondrion but may also be present in glycosomes [9,10]. Likewise comparable with PCF T. brucei, these other trypanosomatids have an elaborate carbohydrate metabolism, including the mitochondrial TCA cycle and they can also convert pyruvate into alanine within glycosomes by an alanine dehydrogenase. As malate dehydrogenase and phosphoenolpyruvate carboxykinase are in the glycosomes, it is likely that they also produce succinate in these organelles, which could balance NAD⁺/NADH like in PCF T. brucei (Fig. 1C) [1]. This is particularly relevant for Leishmania, as its genome lacks the AOX gene and it likely uses host-obtained G3P for gluconeogenesis [11]. Amastigotes of Leishmania spp. are capable of gluconeogenesis, exemplified by the essentiality of glycosomal fructose-1,6-bisphosphatase (FBPase) activity [12]; possibly this holds true also for T. cruzi and PCF T. brucei. For a detailed comparison of glycosomal metabolism in trypanosomatids, see [6,7].

In contrast to most other organisms, activity regulation of hexokinase (HXK) and phosphofructokinase (PFK), by product inhibition or allosteric effectors is virtually absent in trypanosomatids. Without this regulation, the compartmentalization of the first seven enzymes is crucial: it precludes that HXK and PFK are boosted by the cytosolic ATP beyond the capacity of the downstream glycolytic reactions, which would result in accumulation of intermediates [13-16]. However, it is possible that additional regulation occurs. For example, PFK for glycolysis and FBPase for gluconeogenesis are simultaneously expressed in glycosomes, but FBPase is kept silenced under glycolytic conditions to prevent futile cycling. Such regulation may occur through post-translational modifications as a considerable number of glycosomal enzymes are phosphorylated at different sites in BSF and PCF T. brucei [17,18]. This may redirect metabolic fluxes within the glycosomes upon altered nutritional conditions and/or be part of a developmental program (see below).

**Dynamics of glycosomes**

As the kinetoplastids encounter different nutrients during their life cycle, their metabolism and also the glycosomal enzyme content can change dramatically. For example, glycolytic enzymes may constitute >90% of the protein content of glycosomes in BSF T. brucei [19], whereas this drops to 40-50% in PCF parasites that in the tsetse fly’s midgut rarely find glucose and rely heavily on mitochondrial catabolism of amino acids. Even in the presence of glucose, most glycosomal glycolytic enzymes are strongly downregulated in the PCF (Fig.
glycosome proteome enzymes’ developmentally regulated, metabolic phosphatase differentiation, high
phosphatase activity of PIP39 is elevated upon tyrosine phosphorylation, but downregulated when dephosphorylated by PTP1. This self-inactivation loop is interrupted and regulated by the triggers of trypanosome differentiation, CCA. In the presence of CCA, the activation of PTP1 by PIP39 is reduced while PIP39 becomes rapidly activated by specific phosphorylation by an as yet unknown protein kinase after which differentiation is stimulated (Fig. 2D) [27]. Supporting this model, experimental reduction of PIP39 levels in stumpy cells inhibits their transformation to PCFs, consistent with the regulatory role of this phosphatase in the differentiation of T. brucei. Localization studies showed that PIP39 is present in glycosomes, and the removal of its peroxisomal-targeting signal prevented the ability of PIP39 to promote differentiation, highlighting that glycosomes are the important site of action for this phosphatase [27].

Role of glycosomes in life-cycle differentiation
The extensive cellular remodelling during the life-cycle of trypanosomatids (Fig. 2A and B) involves signalling pathways, and the existence of such pathways directed to glycosomes was proposed previously [21,26]. Recent experiments confirmed such a pathway: a phosphatase signalling cascade that regulates an early step in differentiation in T. brucei [27]. In the mammalian bloodstream, trypanosomes differentiate from proliferating long-slender forms to non-dividing short-stumpy forms (Fig. 2B). When taken up by a tsetse fly during a bloodmeal, the stumpy forms differentiate within the insect’s midgut into PCFs (Fig. 2A), whereas the long-slender forms are not able to do so and die [28]. In the stumpy forms, a tyrosine phosphatase (ThPTP1) [29] prevents cells from premature differentiation until its activity is reduced in the presence of the developmental triggers citrate/cis-aconitate (CCA) [30] whose uptake is controlled by the plasma-membrane carboxylate transporter PAD [31]. The downstream target of PTP1 was identified as a DxDxT/V phosphatase, PIP39 [27]. The phosphatase activity of PIP39 is elevated upon tyrosine-phosphorylation, but downregulated when dephosphorylated by PTP1. With the formation of the PIP39-PTP1 complex a bistable regulatory switch is created, as PIP39 reinforces its own repression through increasing the PTP1 activity (Fig. 2C) [27]. This self-inactivation loop is interrupted and regulated by the triggers of trypanosome differentiation, CCA. In the presence of CCA, the activation of PTP1 by PIP39 is reduced while PIP39 becomes rapidly activated by specific phosphorylation by an as yet unknown protein kinase after which differentiation is stimulated (Fig. 2D) [27]. Supporting this model, experimental reduction of PIP39 levels in stumpy cells inhibits their transformation to PCFs, consistent with the regulatory role of this phosphatase in the differentiation of T. brucei. Localization studies showed that PIP39 is present in glycosomes, and the removal of its peroxisomal-targeting signal prevented the ability of PIP39 to promote differentiation, highlighting that glycosomes are the important site of action for this phosphatase [27].

Analysis of phosphoproteome data [18] revealed that many glycosomal proteins, including metabolic enzymes, are phosphorylated. Phosphorylation of these proteins was developmentally regulated, although the role of changing the phosphorylation state on the enzymes’ activities remains to be elucidated. Interestingly, a recent high-confidence glycosome proteome analysis revealed a relatively high number of protein phosphatases, including two serine/threonine phosphatases, PP2C1 and PP2C2, and the two DxDx
phosphatases PIP39A and B. Remarkably, the analysis detected only one protein kinase, PKAC2, together with its putative neurobeacin membrane anchor, suggesting that the PKAC2 is associated with the external surface of glycosomes [32].

In the case of the CCA/PAD/PTP1/PIP39 signalling cascade, the developmental signal is trafficked to glycosomes, where PIP39 becomes inaccessible for the cytosolic PTP1 and thus irreversibly disconnected from the PTP1-PIP39 complex ensuring a one-way commitment for differentiation. Although the target(s) of PIP39 within glycosomes is(are) unknown, developmental effects mediated through metabolic perturbation are already well known, particularly with respect to their impact on surface protein expression [33]. We propose that the compartmentalized developmental signalling molecules transmitted from the cytosol to glycosomes operate via the dephosphorylation of phosphorylated target proteins by glycosomal phosphatases and are subsequently released into the cytosol or degraded in the lysosome during remodelling of the glycosomes.

**Origin and evolution of glycosomes**

Peroxisomes comprise a family of organelles, which are quite heterogeneous in morphology and enzyme content. This heterogeneity is found between different organisms as well as within organisms, dependent on their environment or developmental stage. However, all family members have in common some morphological features, such as a single boundary membrane, the presence of various specific peroxisomal enzyme systems and the mode of biogenesis. The peculiar way by which these organelles proliferate has been reviewed extensively elsewhere [34-38].

Peroxisomes can be found in representatives of all major branches of eukaryotes [39] and therefore must have already been present in the Last Eukaryotic Common Ancestor (LECA) and lost in lineages to those extant species from which the organelles are absent [5]. Kinetoplastid glycosomes are peculiar by possessing enzymes of the glycolytic pathway and other core metabolic processes not found in peroxisomes of other organisms. In contrast, typical peroxisomal enzymes such as \( \text{H}_2\text{O}_2 \)-producing oxidases and catalase are not found in glycosomes. Since aldolase has also been detected in peroxisome-like organelles of a *Diplonema* species [40], but no peroxisomal glycolytic enzymes were found in *Euglena* and outside the Euglenozoa, glycosomes must have originated in a common ancestor of the Kinetoplastea and Diplonemida lineages, after their separation from the Euglenida branch (Fig. 3) [5]. Most likely, glycolytic and some other enzymes of intermediary metabolism became sequestered into peroxisomes of this ancestor due to minor mistargeting of proteins, as generally observed in eukaryotic cells [41]. This compartmentalization must then have been preserved and expanded when it provided a selective advantage. Since the discovery of glycosomes in 1977 [42], there have been several hypotheses about the function of this compartmentalization. These hypotheses range from facilitating a high glycolytic flux [19,43,44] – a hypothesis which has been refuted – to compensating for lack of activity regulation of enzymes such as HXK and PFK [13] – a function that has been proven for present-day glycosomes [14-16], but is unlikely the original one [5]. A current notion is that the compartmentalization provided an increased metabolic flexibility to the ancestral cells and thus the ability for a more efficient adaptation to varying environmental conditions. During further evolution, glycosomes of cells in different kinetoplastid lineages acquired additional enzymes and pathways and lost others [5].

**Glycosomes and parasitism**

The origin of the glycosome is estimated to have occurred in a free-living protist
approximately 600 million years ago (Mya), in the period that metazoans originated (Fig. 3). Therefore, the origin and early evolution of glycosomes was unrelated to parasitism; hosts of extant trypanosomatids such as mammals and insects appeared only approximately 200 and 300 Mya, and annelids, arthropods, fish and land plants between 500 and 400 Mya [5].

As mentioned above, a crucial advantage of having glycosomes seems the capacity for rapid and efficient turnover of the specifically compartmentalized metabolic units when environmental conditions change. Having acquired the glycosomes, kinetoplastids may have, at some later stage, exploited them for development of an efficacious parasitic life style. Parasitism evolved multiple times within the Kinetoplastea (Fig. 3) [45,46]. Probably, free-living aquatic kinetoplastids initially infected insects and subsequently additional hosts such as vertebrates and plants were also parasitized. For each of these steps the kinetoplastid organism should likely have possessed a metabolic flexibility to adapt to sudden, drastic changes of environments; it had to develop a life-cycle, the monoxenous (one host) parasite involving stages in an aquatic environment and an insect, the dixenous parasites stages in an insect and a secondary host. Moreover, many parasites have subsequently elaborated their life cycle by occupying different places in the bodies of their hosts, each time exposing them to a different milieu: in the insect initially on the mouth parts and subsequently often also in the digestive system and salivary glands, in the vertebrates extracellularly in the skin and/or haemolymphatic circulation and subsequently often also intracellularly, in different cell types. It could be imagined that the metabolic flexibility offered by the possession of the easily replaceable glycosomes may have contributed to the development of elaborate parasitic life cycles in so many lineages of the Kinetoplastea. Conversely, the enzymatic content of glycosomes in extant trypanosomatids will have been shaped by the specific nutritional conditions successively encountered during their parasitic life cycles. Furthermore, the finding of an involvement of glycosomes in the trypanosome’s signalling system for differentiation suggests another way in which glycosomes may have facilitated development of parasitism in the kinetoplastids.

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References and recommended reading

* of special interest
** of outstanding interest


4. Colasante C, Ellis M, Ruppert T, Voncken F: **Comparative proteomics of glycosomes from bloodstream form and procyclic culture form Trypanosoma brucei brucei.** *Proteomics* 2006, **6**:3275-3293.


** A review that discusses how peroxisomes may have acquired glycolysis to give rise to glycosomes, what might have been the selective advantage, how subsequently, during hundreds of millions of years, other enzymes or pathways may have been sequestered or lost in different lineages of the Kinetoplastea and why the compartmentalization of core metabolism may have facilitated the development of parasitism in these organisms.


* This is a very detailed review of all metabolic processes identified in glycosomes of trypanosomatid parasites, the identification of glycosomal enzymes and other proteins as anti-parasite drug targets and the current state of drug discovery.


* This paper provides experimental data how procyclic *T. brucei* maintains the glycosomal ATP/ADP balance under different nutritional conditions.


12. Naderer T, Ellis MA, Sernee MF, De Souza DP, Curtis J, Handman E, McConville MJ:


* The global quantitative SILAC-labelling phosphoproteomic study of *Trypanosoma brucei* reported in this paper reveals that differential protein phosphorylation is widespread between the different life-cycle stage forms, with also important intraprotein differential phosphorylation. Many glycosomal proteins were also found to be phosphorylated. Phosphorylation may play an important role in regulation of both metabolism and expression, compensating for absence of other kinds of regulatory processes as present in other organisms.


* The authors of this paper describe how the selective autophagy process of pexophagy is used for vacuolar/lysosomal degradation of peroxisomes that are damaged or have become superfluous because of altered physiological situations, when environmental conditions change, during cell differentiation, etc. Mechanistically, pexophagy relies heavily on the cell’s core autophagic machinery, for either macro- or microautophagy, but in recent years a number of auxiliary pexophagy factors have been identified which are important for cargo selectivity.


* This paper describes the identification of the DxDxT phosphatase PIP39 as a crucial T. brucei differentiation signalling molecule, acting downstream of the earlier, by the same authors, discovered PTP1 phosphatase. The data support the notion of a phosphatase signalling cascade in which the developmental signal, via PIP39, is trafficked from the cytosol to the glycosomes. This is the first report of a characterized environmental signalling pathway targeted directly to a peroxisome-like organelle in any eukaryotic cell.


** This excellent paper describes the identification of a carboxylate-transporter family (PAD) required for perception of extracellular citrate or cis-aconitate, the differentiation signal to which trypanosomes respond when transmitted to the tsetse fly. These transporters are expressed on the surface of the transmission-competent stumpy-form bloodstream parasites and at least one member (PAD2) is thermoregulated, showing elevated expression and surface access at the lowered temperature encountered in the fly.

* This article presents a high-confidence quantitative SILAC based proteome of the matrix and membrane of epitope-tagged, magnetic beads enriched glycosomes from procyclic *T. brucei*.


** This is an excellent up-to-date review of peroxisomes. It describes the various peroxisomal reactions that are tightly regulated to adapt to the changing needs of the cell and varying external environments and how, accordingly, these organelles are remarkably fluid and can change dramatically in abundance, size, shape and content in response to numerous cues. These dynamics are controlled by multiple aspects of peroxisome biogenesis, discussed in detail, that are coordinately regulated with each other and with other cellular processes.


* This recent minireview summarizes the significant progress made in the last few years towards the understanding of the different aspects of peroxisome formation: the sorting of peroxisomal membrane proteins, matrix-protein import and multiplication of the organelles. Conflicting models of peroxisome assembly are discussed and gaps in the current knowledge indicated.


41. Martin W: Evolutionary origins of metabolic compartmentalization in eukaryotes.
An evolutionary intriguing question is how an entire pathway can be transferred from one compartment of a eukaryotic cell to another one over evolutionary time; pathway enzymes acquire their targeting signals for the new compartment individually, not in unison; however, newly routed individual enzymes are useless. The author suggests that pathways attain novel compartmentalization variants via minor mistargeting. If protein targeting possesses enough imperfection such that small amounts of entire pathways continuously enter novel compartments, selectable units of biochemical function would exist in new compartments.


Figure legends

Figure 1. (A) Overview of glucose metabolism in Trypanosomatidae. Solid lines represent the major glycolytic routes that are expressed in BSF *T. brucei* and in most developmental stages of all trypanosomatid parasites, dashed lines are extensions that can be present dependent on environmental conditions in PCF *T. brucei* (and possibly in other trypanosomatids) and under specific environmental conditions also in BSF cells [47]. Glycosomal β-oxidation and alanine production in glycosomes of *T. cruzi* (and possibly PCF *T. brucei*) are not depicted. The numbered enzymes are also in panel D. (B) Schematic depiction of the glycolytic branches that are used in BSF *T. brucei* in the presence or absence of oxygen. (C) Schematic depiction of the glycolytic branches that are used in PCF *T. brucei* (and other trypanosomatid parasites). Solid lines represent dominant branches, dashed lines represent pathways that are downregulated, but can be used with glucose as a carbon source. (D) An overview of the quantitative differences in enzyme levels and activities of glycolytic enzymes in PCF as compared to BSF trypanosomes. The activities of different enzymes were measured at similar temperatures within each study. **a** Strain EATRO 110, measured in purified glycosomes at 37°C [48]; **b** strain 427, values for activities in homogenate are given, but glycosomal fraction gave similar results [49]; **c** strain 427; BSF values were measured at 25°C [21], PCF values were at 28°C [50]; **d** de Duve Institute; P. Michels and F. Oppendoes unpublished for strain 427; **e** authors noted that activity in BSF is low due to endogenous inhibitor; the value for the glycosomal fractions is −97%, which suggests that it is a cytosolic inhibitor; **f** this enzyme is cytosolic in the PCF stage; **g** not detectable in BSF [51].

Figure 2. Regulation of *Trypanosoma brucei* differentiation by a phosphatase cascade. (A and B) Simplified life cycle of *T. brucei*. (1) Proliferating long-slender bloodstream-form; (2) short-stumpy form in the mammalian bloodstream; (3) short-stumpy form in tsetse fly midgut; (4) proliferating procyclic form in tsetse midgut. The developmental stages between procyclics and long-senders (e.g. epimastigotes, metacyclic trypanastigotes in the tsetse salivary) have not been included. (C) Stumpy-form trypanosomes in the bloodstream. In mammalian blood, the transmissible stumpy forms express only the PAD1 transporter on their cell surface, while PAD2 is presented on the surface of intracellular vesicles (1). PIP39 is activated upon tyrosine-phosphorylation by an unknown kinase and dephosphorylated (inactivated) by PTP1 (2). In the assembled PTP1/PIP39 complex, PIP39 increases the enzyme activity of PTP1, reinforcing its own repression (3). The active PTP1 acts as a molecular brake stopping the premature differentiation of stumpy forms (4). (D) Stumpy-form trypanosomes in the tsetse midgut. In the tsetse midgut, with the drop of temperature, PAD2 protein expression is upregulated and the protein translocates to the cell surface (5). The increased number of transporters on the cell surface enables highly efficient CCA transport into the cell even at very low extra-cellular concentrations. In the cell CCA disrupts the PTP1/PIP39 complex, which leads to the downregulation of PTP1 activity, rapid tyrosine phosphorylation of PIP39 (6) and the initiation of differentiation (7). After the breakdown of the PTP1/PIP39 complex the PIP39 translocates to the glycosomes and becomes inaccessible to PTP1, ensuring the irreversibility of differentiation (8).

Figure 3. Glycosome evolution and development of parasitism within organisms belonging to the clade of euglenozoan protists. Phylogenetic relationships of the organisms are shown at an approximate evolutionary time scale. Underneath is shown the time scale (in Mya, millions of years ago) at which major taxons arose of which representaties act as hosts to the extant parasites indicated at the right hand side. Peroxisomes (P) must already have been present in the last eukaryotic common ancestor (LECA); the ancestral organism that acquired
glycosomes (G) by compartmentalizing glycolysis in its peroxisomes is indicated. Parasitism has arisen independently, multiple times in the Kinetoplastea. Names of organisms that are free-living, monoxenous parasites (a life cycle with a single host) or dixenous parasites (life cycle involving two hosts) are indicated in red, green and blue, respectively. Asterisks indicate the approximate timing at which, in different lineages, ancestral kinetoplastid organisms may have infected a new host lineage, giving either rise to a monogenetic or a digenetic parasitic life cycle of the organisms. The time of the branchpoint of the genera within the Prokinetoplastina is arbitrarily chosen and remains to be established. Further details can be found in [5,46].
Figure 1
Figure 2
Figure 3