Molecular & Biochemical Parasitology 196 (2014) 61–70

Contents lists available at ScienceDirect

Molecular & Biochemical Parasitology

Review

Acquisition and biosynthesis of saturated and unsaturated fatty acids by trypanosomatids

Antonio D. Uttaro

Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina

ARTICLE INFO

Article history:
Received 22 November 2013
Received in revised form 28 March 2014
Accepted 1 April 2014
Available online 13 April 2014

Keywords:
Trypanosoma
Leishmania
Fatty acid
Precursor
Synthesis
Scavenging

ABSTRACT

As components of phospholipids and glycosylphosphatidylinositol anchors, fatty acids are responsible for forming the core of biological membranes and the correct localization of proteins within membranes. They also contribute to anchoring proteins by direct acylation of specific amino acids. Fatty acids can be used as energy sources and serve as signaling molecules or precursors for their synthesis. All these processes highlight the important role of fatty acids in cell physiology, justifying the diverse strategies for their acquisition evolved by different organisms. This review describes several recent findings in the salvage and biosynthesis of fatty acids by parasitic protists belonging to the class Kinetoplastea. They include two biosynthetic routes, the mitochondrial one and a peculiar membrane-associated pathway, the synthesis of polyunsaturated fatty acids, and the scavenging of lysophospholipids and lipoproteins from host plasma. These different processes are also explored as putative targets for chemotherapy.

Contents

1. Introduction

Protists belonging to the Trypanosomatidae family, such as *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania* spp., are parasitic flagellates responsible for several human and animal diseases, like Chagas' disease, sleeping sickness and leishmaniases. Together, they threaten over 400 million people, with 30 million currently being infected, mainly distributed in underdeveloped countries. These neglected diseases are often fatal if not treated [1].

For years, trypanosomatids have been considered as a source of rarities, having a number of diverse, unusual biological features like aerobic fermentation, with glycolysis compartmentalized in organelles named glycosomes [2], editing of mitochondrially encoded pre-mRNAs, transsplicing of nuclear transcripts and antigenic variation [3]. These peculiarities were attributed to the supposed early evolutionary branching of these organisms from the main trunk of the eukaryotic tree, considering them as primitive characters. Current opinion holds that this last concept is most probably incorrect; most of these processes are more easily explained by considering them as acquisitions by which the ancestral kinetoplastids adapted to their environment and which were elaborated when later these flagellates adapted parasitic life styles.

Fatty acid (FA) acquisition by trypanosomatids can equally be considered as a process with peculiarities that resulted from adaptation to the parasitic conditions. In the 1970s it was found that trypanosomatids had an apparently low or even null capacity for *de novo* FA biosynthesis, which was in agreement with the metabolic

E-mail address: toniuttaro@yahoo.com.ar

http://dx.doi.org/10.1016/j.molbiopara.2014.04.001
0166-6851/

dependence expected for parasites. Interestingly, instead of losing the biosynthetic capacity, the parasites had evolved new strategies to take up lipids from their environmental milieu as well as established a novel metabolic pathway for *de novo* synthesis of FAs. The nature of this pathway, only recently elucidated in its unexpected details, had provoked decades of disconcert in the scientific community. As the features of fatty acid biosynthesis in the trypanosomatids are different enough from those present in mammals, they reveal potential for development of antiparasitic drugs.

This review highlights the recent findings related to the scavenging and synthesis of FAs by trypanosomatids. Excellent reviews have previously been published by others, dealing with the acquisition of sterols [4], sphingolipids [5,6], phospholipids [5,7–9] and FAs [4,9,10]. Finally, I hypothesize about the origin of precursors for FA synthesis, based on recent findings about the catabolism of energy sources and analysis of the information available in trypanosomatid genome databases.

2. Scavenging of lipids as fatty acid source

Trypanosomatids have evolved multiple ways to scavenge lipids from their hosts. Plasma lipoproteins like LDL represent a main source of sterols and phospholipids. These parasites are able to internalize lipoproteins by receptor-mediated endocytosis [11–13]. Once endocytosed, the LDL receptor is recycled and LDL-derived apolipoprotein B degraded in lysosomes, releasing steryl esters and phospholipids. These lipids are then hydrolyzed, providing FAs, sterols and phospholipid head groups to the cell (Fig. 1) [14,15]. Another important source of lipids are HDL particles, which are also receptor-mediated endocytosed by *T. brucei* [16]. Interestingly, a subtype of human HDL is lytic for the *T. brucei brucei* subspecies [17], whereas *T. b. rhodesiense* and *T. b. gambiense* have developed resistance mechanisms against its trypanolytic factors [18,19].

Bloodstream forms of *T. brucei* have an additional way for acquiring FAs and phospholipids head groups by scavenging the relatively abundant lysophosphatidylcholine, which is present at hundreds of micromolar concentrations in host plasma [20]. It is performed through a pathway consisting of three enzymes, phospholipase A1, acyl-CoA synthetase and lysophosphatidylcholine:acyl CoA acyltransferase [21]. Phospholipase A1 generates FAs from exogenous lysophosphatidylcholine which are channeled to the synthetase, producing acyl-CoA. The acyl-CoA formed by this route is in a separate pool from that derived from exogenous free FAs and could be alternatively used by the acyltransferase to acylate a second molecule of exogenous lysophospholipid, providing phosphatidylcholine to the cell (Fig. 1).

Although free FAs are present in low amounts in plasma [22], they are readily taken up by kinetoplastid protists [23,24]. The uptake presents a biphasic course, with an initial rapid phase. Depending on the FA and parasite species, 50–90% of FA incorporation occurs during the first 15 s of the radiolabeling experiment. It is followed by a slower accumulation phase. The initial phase is thought to represent the binding of FAs to the cell surface, which is mediated by serum albumin in a reversible process (Fig. 1). Supporting this hypothesis, the release of preloaded FAs from *T. brucei* cells is stimulated by a direct interaction with free albumin [23]. In mammalian cells, the binding of FA to the cell surface is assisted by the plasma membrane FA binding protein (FABPpm) and glycoprotein CD36. Whereas there are no obvious orthologs of CD36 in the trypanosomatid' genome, FABPpm shows high amino acid identity to kinetoplastid' mitochondrial aspartate aminotransferases. FABPpm has a dual function and localization in mammalian cells. It localizes at the mitochondria having aspartate aminotransferase activity as well. In addition, FABPpm was also found on the cell surface of several tissues [22]. Whether the corresponding trypanosomatid proteins have a dual localization or are involved in FA uptake is not known.

Once at the cell surface, FAs can cross the plasma membrane by simple (passive) diffusion, or be translocated by membrane-associated FA transport proteins (FATPs, Fig. 1) [22,25]. In a similarity search using yeast Fat1p or human FATP1 amino-acid sequences as queries, we identified orthologs in the genome of *T. cruzi* (TriTrypDB identification number TcCLB.511907.110) and *Leishmania major* (LmjF.24.1780), but not in that of *T. brucei* (Table 1). These are large proteins (1064 and 1311 amino acids, respectively) compared to Fat1p (623 amino acids) but share important similarities, particularly in the region supposed to be involved in FA recognition. This difference between *T. brucei* and other trypanosomatids is intriguing, but could be related to the extracellular condition of the former species, while the other parasites live predominantly intracellularly in mammals. If the proteins identified above are *bona fide* translocators, it could be possible that they are needed in the uptake of free FAs which are found in very low amounts within the host cell, whereas *T. brucei* replaced FATPs by a system to more efficiently rely on the relatively abundant lysophospholipids found in plasma (see above). Another possibility is that FATPs are involved in the uptake of very long chain FAs (VLFAs, Fig. 1), which are used in the synthesis of glycosylphosphatidylinositol (GPI) anchors of *T. cruzi* and *Leishmania* spp. but not in *T. brucei* GPI [10]. Although the former parasites are able to *de novo* synthesize C24 and C26 VLFAs (see Section 3), the uptake would contribute to assure enough VLFAs for the correct localization of essential macromolecules on the cell surface *via* GPI anchoring.

Internalized FAs are activated to their CoA thioesters by acyl-CoA synthetases (ACS), trapping them inside the cell. This acylation represents a vectorial transport of FAs which are then available for subsequent metabolism [22,25]. Trypanosomatids have numerous putative ACSs. *T. brucei* has five synthetases (ACS1–5) [7,26] sharing 26–28% identity with the yeast ACS FAA1 and other two putative ones (Tb927.11.4490 and Tb927.11.7530, Table 1) distantly related (less than 15% identity). TbACS1–4 are encoded on chromosome 9 in a tandem array, whereas TbACS5 is encoded on chromosome 10. TbACS1 showed preference for polyunsaturated fatty acids of 18–22 carbons (C18–22 PUFAs) and C10–14 saturated FAs [26]. TbACS2 was only active on short saturated FAs (C6–12), whereas TbACS3–4 were active on saturated C8–18 FAs as well as unsaturated C16–20 FAs [26]. TbACS5 showed preference for the saturated FA myristic acid (14:0) [7]. They usually are membrane associated proteins but their subcellular localization in these parasites was not determined. It is not known which of these ACSs is involved in the scavenging of lysophosphatidylcholine described above. *Saccharomyces cerevisiae* internalizes PUFAs inefficiently, but the expression of TbACS1 stimulated two- to sixfold the incorporation of exogenous C20–22 PUFAs into the yeast [Tripodi and Uttaro, unpublished results]. TbACS1was potentially expressed at the plasma membrane, interacting with endogenous yeast FATP (Fat1p), and then mediating a vectorial transport. Co-expression of TbACS1 and trypanosomatid FA desaturases and elongases (Sections 3 and 4), allowed us to notably increase these heterologous activities using exogenous FA substrates, facilitating their biochemical characterization. It is expected that equivalent interactions between ACSs and FATPs also occur in trypanosomatids.

Acyl-CoAs usually bind to proteins (acyl-CoA-binding proteins or ACBPs) to prevent their metabolism as free acyl-CoAs and allow their efficient shuttling to the cell acylation machinery. An ACBP (Table 1) was isolated and characterized in *T. brucei*, which showed preference for saturated C10-14 acyl-CoAs. Its presence stimulated the incorporation of myristate from myristoyl-CoA into GPI, but not into neutral lipids or phospholipids, in a cell-free assay [27].

Table 1
Names and TriTrypDB identification numbers of characterized and hypothetical proteins involved in transport, synthesis and modification of fatty acids.

Protein	Locus			Reference
	T. brucei	*T. cruzi*	*L. major*	
Fatty acid translocator protein (FATP)		TcCLB.511907.110	LmjF.24.1780	
Acyl-CoA synthase	Tb927.9.4190 (ACS1)	na[a]	na	[26]
	Tb927.9.4200 (ACS2)			
	Tb927.9.4210 (ACS3)			
	Tb927.9.4230 (ACS4)			
	Tb927.10.3260 (ACS5)			[7]
	Tb927.11.4490			
	Tb927.11.7530			
Acyl-CoA binding protein (ACBP)	Tb927.4.2010	na	na	[27]
ELO system				
Ketoacyl-CoA synthase (KS or Elo)				
Elo1	Tb927.7.4180	TcCLB.511245.130	LmjF.14.0640	[42]
		TcCLB.506661.30	LmjF.14.0650	
			LmjF.14.0660	
Elo2	Tb927.7.4170	TcCLB.511245.140	LmjF.14.0670	
		TcCLB.506661.20		
Elo3	Tb927.7.4160	TcCLB.511245.150	LmjF.14.0680	
		TcCLB.506661.10	LmjF.14.0690	
			LmjF.14.0700	
			LmjF.14.0705	
Elo4		TcCLB.511245.160	LmjF.14.0710	
		TcCLB.510989.10	LmjF.14.0720	
			LmjF.14.0730	
			LmjF.14.0740	
Elo5	Tb927.5.4530	TcCLB.509539.30	LmjF.05.1170	
		TcCLB.506717.10		
Elo6			LmjF.32.1160	
Enoyl-CoA reductase	Tb927.3.1840	TcCLB.437545.10	LmjF.25.1770	[41]
Fatty acid synthase system (FAS II)				
ACP	Tb927.3.860	TcCLB.511867.140	LmjF.27.0290	[33–35]
Ketoacyl-ACP synthase (KS)	Tb927.2.3910	TcCLB.511109.120	LmjF.33.2720	
Ketoacyl-ACP reductase (KR)	Tb927.2.5210	TcCLB.511627.150	LmjF.27.2440	
Hydroxyacyl-ACP dehydratase (DH)	Tb927.8.1440	TcCLB.508547.50	LmjF.07.0430	
			LmjF.07.0440	
Enoyl-ACP reductase (ER)	Tb927.7.7410	TcCLB.510609.50	LmjF.05.0520	
	Tb927.9.7190		LmjF.04.0290	
Fatty acid desaturases				
Type I				
Δ9	Tb927.8.6000	TcCLB.509239.10	LmjF.24.2250	[51]
		TcCLB.511075.9	LmjF.14.0510	
Type II				
Δ12	Tb927.2.3080	TcCLB.429257.20	LmjF.33.3270	[54,55,60]
		TcCLB.508737.70		
Δ15			LmjF.10.0010	[55]
			LmjF.10.1320	
Type III				
Δ6			LmjF.36.6950	[62]
Δ5			LmjF.07.1090	[62]
Δ4	Tb927.10.7100	TcCLB.507609.40	LmjF.14.1340	[62]
		TcCLB.510181.20		
Acetyl-CoA carboxylase (ACC)	Tb927.8.7100	TcCLB.510599.14	LmjF.31.2970	[63]
		TcCLB.508369.50		
Acetyl-CoA synthetase	Tb927.8.2520	TcCLB.504427.110	LmjF.23.0540	[64]
		TcCLB.509331.30	LmjF.23.0710	
Threonine dehydrogenase (TDH)	Tb927.6.2790	TcCLB.507923.10		[66]
		TcCLB.511753.120		
2-amino-2-ketobutyryl-CoA ligase	Tb927.8.6060	TcCLB.511071.140		[66]
		TcCLB.511899.40		
Acetyl-CoA thioesterase (ACH)	Tb927.3.4260		LmjF.29.1690	[73]
Acetate:succinate CoA trasferase (ASCT)	Tb927.11.2690	TcCLB.504153.360	LmjF.33.2340	[72]
		TcCLB.506301.50		
Thiolase (SCP2 type)	Tb927.8.2540	TcCLB.510507.20	LmjF.23.0690	[70]
		TcCLB.509463.30		
3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthetase	Tb927.8.6110	TcCLB.511071.50	LmjF.24.2110	[69]
		TcCLB.511903.40		
HMG-CoA reductase	Tb927.6.4540	TcCLB.509167.20	LmjF.30.3190	[69]
		TcCLB.506831.40		
HMG-CoA lyase	Tb927.4.2700	TcCLB.506635.80		
Hydroxybutyrate dehydrogenase (HBDH)	Tb927.10.11930	TcCLB.506567.70		[77]
		TcCLB.507049.60		

[a] Not analyzed.

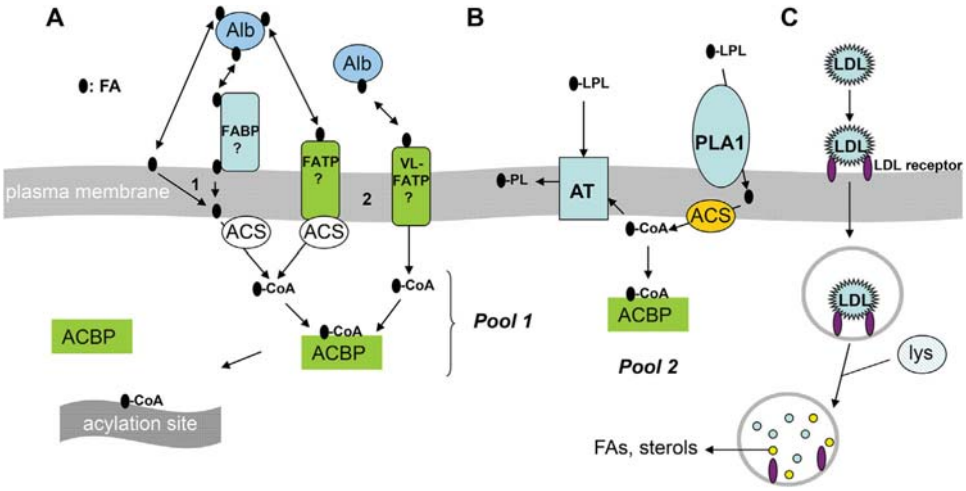

Fig. 1. Sources of fatty acids for trypanosomatids. (A) Free fatty acids (FA), attached to serum albumin (Alb), bind to the parasite cell surface. This binding could be assisted by putative FA binding proteins (FABP). FA can enter the cell by passive diffusion (1) or translocation *via* a FA transport protein (FATP) (2). Vectorial acylation by acyl-CoA synthetases (ACS) activates and trap FA inside the cell. Acyl-CoA binding proteins (ACBP) bind acyl-CoA and transport them to the acylation sites. Very long chain FA could be translocated by specialized FATP (VL-FATP) with simultaneous acylation. (B) Lysophospholipids (LPL) are hydrolyzed by phospholipase A1 (PLA1) providing FA to an unknown ACS. Resultant acyl-CoAs are available to the cell as a different pool to that obtained from free FA. These acyl-CoA can alternatively be used to acylate exogenous LPL by LPL:acyl CoA acyltransferase (AT) providing phospholipids (PL) to the cell. (C) Lipoproteins (LDL) are receptor-mediated endocytosed. Receptors are recycled and LDL degraded, releasing sterols and FA (lys, lysosome).

Interestingly, ACBP appears to be essential for bloodstream form *T. brucei* [28].

3. Synthesis of saturated fatty acids

FAs are biosynthesized by the successive addition of two carbon units to a growing carboxylic acid chain. The carboxylic chain is esterified *via* the pantothenyl group to the acyl carrier protein (ACP). The biosynthesis involves four basic enzymatic reactions carried out by a soluble complex named FA synthetase (FAS). The first reaction is the condensation of acetyl-CoA (primer) and malonyl-ACP (two-carbon donor), producing acetoacetyl-ACP by β-ketoacyl-ACP synthase (KS) or condensing enzyme. Acetoacetyl-ACP is reduced to β-hydroxybutyryl-ACP by β-ketoacyl-ACP reductase (KR), followed by its dehydration to *trans*-2-butenoyl-ACP, catalyzed by β-hydroxyacyl-ACP dehydratase (DH). Finally, the double bond is reduced by enoyl-ACP reductase (ER) producing butyryl-ACP, which initiates a new cycle of four reactions [29]. In prokaryotes, several cycles and the use of different primers conduct the synthesis of even- and odd-numbered and branched C15–19 FAs, by a complex of numerous monofunctional enzymes named FAS II, including the four described above and illustrated in Fig. 2. Similar complexes are found in eukaryotic organelles from prokaryotic origin: *i.e.* mitochondria and plastids. Additionally, eukaryotes possess a cytoplasmic complex (FAS I) formed by one or two large multidomain enzymes, responsible for the bulk C16–18 saturated FAs of the cell [29]. Longer FAs are synthesized by an independent elongating system (ELO), associated with the endoplasmic reticulum membrane [29,30]. Interestingly, ELO synthesizes FAs of up to 26 carbons by means of the same four reactions described above, but using acyl-CoA (usually starting from myristoyl- or palmitoyl-CoA) and malonyl-CoA as substrates. Each of the four reactions is carried out by a distinct enzyme, which are all structurally or phylogenetically unrelated to those of FAS, representing a good example of evolutionary convergence (Fig. 2). Elo is also used to name the condensing or elongating enzyme of this system, equivalent to the KS of FAS [30].

Trypanosomatids have long been considered as organisms with a low capacity for FA biosynthesis [31]. They were observed to incorporate labeled acetate and carbon derived from glucose and glycerol into FAs, apparently by elongating existing long chain FAs. Bloodstream form *T. brucei* cells did not incorporate significant label, previously taken as suggestion for the complete lack of FA biosynthesis in this stage [32]. However, this notion made it difficult to understand how this life-cycle form could satisfy the paramount amounts of long chain FAs needed for phospholipids' biosynthesis and myristate for the synthesis of GPI anchors for the variable surface glycoproteins (VSG), especially during a high parasitemia. Free FAs are readily salvaged but their concentrations in plasma are low. Even considering that these are also available as

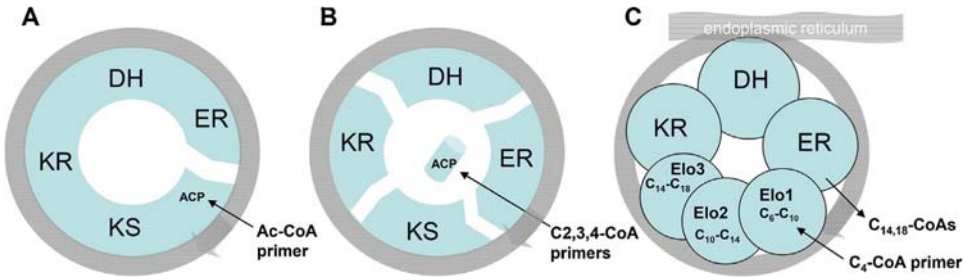

Fig. 2. Schematic representation of the three known systems for *de novo* saturated fatty acid biosynthesis. (A) FAS I, (B) FAS II and (C) ELO. Only relevant domains or polypeptides are shown: Ac-CoA, acetyl-coenzyme A primer; ACP, acyl carrier protein; DH, hydroxyacyl dehydratase; Elo1–3, KS isozymes of ELO system; ER, enoyl reductase; KR, ketoacyl reductase; KS, ketoacyl synthase.

lysophosphatidylcholine, they could not satisfy the requirements of the parasite population.

Trypanosomatid genomes do not show indications for the presence of FAS I, but encode FAS II enzymes and ACP (Table 1), all of them predicted to localize in the mitochondrion. This localization was later experimentally confirmed and each enzyme functionally characterized in *T. brucei* [33,34] and *L. major* [35]. The system is involved in octanoate (8:0) and palmitate (16:0) synthesis [33]. Octanoate is the precursor of lipoate which, as lipoamide, is a cofactor of several mitochondrial dehydrogenases [36], whereas palmitate is used in the synthesis of mitochondrial phospholipids. RNA interference (RNAi) or genomic deletion of ACP indicated that this pathway is essential for both the insect and bloodstream life cycle stages of the parasite, and exogenous FAs did not bypass the lack of ACP. FAS II contributes only 10% of the total FA biosynthesis of the cell (see below) [33]. It suggests that the incapacity of the mutant to provide the cell with locally synthesized short and medium chain FAs is not the reason of the deleterious effect, but a consequence of a massive alteration of mitochondrial functions. Actually, respiratory inhibition, alteration of mitochondrial ultrastructure and changes in phospholipid composition were found following ACP RNAi [37]. In addition, it caused loss of the mitochondrial genome in *T. brucei* bloodstream forms [38]. The primary effect of ACP depletion appeared to be the alteration of phospholipid composition of the mitochondrial membrane which affected the assembly of respiratory-chain complexes and the tripartite attachment complex involved in kinetoplast DNA segregation.

From where come the FAs needed to supply the massive requirements of the cells in order to synthesize GPIs and other membrane components, such as phospholipids? As described above, salvage and mitochondrial synthesis *via* FAS II are not enough. The answer came from Paul Englund and coworkers, who invested several years to solve what they named the "myristate mystery" [39]. As a result of their studies on the biosynthesis and FA remodeling of GPI, they observed efficient FA synthesis in a cell-free extract of bloodstream form *T. brucei* [40]. Contrary to expectation, the synthesis was associated to membranes, used malonyl-CoA as two-carbon donor and butyryl-CoA as primer, while acetyl-CoA appeared unable to prime. The major product using extracts from the bloodstream stage was myristoyl-CoA which was efficiently used for GPI remodeling in the same cell-free preparation, indicating that both pathways were in the same compartment, most probably in the endoplasmic reticulum. The major product using extracts from the insect stage was stearoyl-CoA (C18:0).

An *in silico* survey made after the completion of the trypanosomatid genomes sequencing detected multiple Elo-like KS genes (Table 1). A phylogenetic and synthenic analysis of them allowed the prediction and classification of Elos involved in the elongation of saturated and unsaturated FAs [41,42]. *T. brucei* has a cluster of three genes on chromosome 7, TbElo1-3, which were characterized by Englund' group as being responsible of the FA biosynthesis described above [41]. Several findings help them to solve the puzzle. Thiolactomycin, an inhibitor of prokaryotic KS, kills the parasites in culture and completely inhibited the synthesis of myristate in cell-free systems. Cerulenin, another KS inhibitor, blocked the elongation of decanoate (C10:0) to myristate, but did not affect the extension of the butyrate (C4:0) primer to decanoate [40]. These data indicated that more than one membrane-associated KS-like enzyme was present in trypanosomes, showing Elos as plausible candidates. ACP RNAi did not inhibit FA synthesis in the cell-free system, ruling out the participation of FAS II. Using membranes from bloodstream form *T. brucei* in which TbElo1 was knocked out, synthesis from primers ranging from C4- to C12-CoA was almost completely lost, whereas synthesis from C14- and C16-CoA was less affected. By using membranes from each Elo mutant, it was possible to show that trypanosomes have a modular system for FA synthesis, with Elo1 elongating C4- to C10-CoA, Elo2 elongating C10- to C14-CoA and Elo3 elongating C14- up to C18-CoA. This modular synthesis allows the parasite to regulate the production of different intermediates as required throughout its life cycle. Expression of Elo3 is downregulated by exogenous FAs, such as those obtainable from the host bloodstream, leaving the product of Elo2, myristoyl-CoA, available for GPI synthesis [41].

T. cruzi has a cluster of four Elo genes, TcElo1-4, with TcElo4 having higher similarity to TbElo3 and TcElo3, probably as a result of a duplication of an ancestral Elo3 gene in the trypanosomatid lineage [42]. TcElo1–3 are assumed to have equivalent functions to the *T. brucei* orthologs [41]. In *S. cerevisiae*, the *ScElo2*Δ/*ScElo3*Δ double mutation is lethal, due to the incapacity of the yeast to synthesize FAs longer than C18 [43]. Expression of TcElo4 rescued this lethality and the VLFA profile of the transfected yeast was quite similar to that of *T. cruzi* epimastigotes [Livore and Uttaro, manuscript in preparation]. This indicates that TcElo4 is responsible for C22-26 VLFAs synthesis in this parasite. In *Leishmania* spp. the situation is more complex. Several duplication events have given rise to larger clusters on chromosome 14. *L. major* has a tandem array comprised of 12 Elo genes and two pseudogenes [42]. Preliminary results indicate that LmElo4.1 and LmElo4.4 are involved in the extension of C18:0/20:0 to C22:0 and C18:0 to C20:0, respectively [Livore and Uttaro, unpublished]. Such Elo diversity in *Leishmania* species could be related to the diversity of VLFAs and fatty alcohols synthesized by these protists. Several Elos with redundant activities but different subcellular localizations could be needed to provide VLFA substrates to other pathways, as for example the pathway involved in the synthesis of fatty alcohols, which is located in glycosomes [44], or synthesis and modification of GPI and other membrane components, located at the endoplasmic reticulum and beyond in the secretory pathway. Another possibility could be that, instead of being able to catalyze several cycles of elongations, each leishmanial Elo is responsible for carrying out only one cycle.

RNAi silencing of enoyl-CoA reductase of the ELO system caused growth arrest in the insect stage of *T. brucei*, but normal growth was restored when the culture was supplemented with C18:0. Elo knockout lines of the bloodstream stage grew normally in culture and were competent for infection in rats. This last result suggests that the ELO system may not be required for growth in lipid-rich environments [41].

4. Synthesis of polyunsaturated fatty acids

Trypanosomatids show several differences with respect to the FA composition found in the plasma of their mammalian host, already early suggesting that these parasites closely regulate their FA composition. Trypanosomes possess a higher proportion of linoleate (C18:2Δ9,12) and other PUFAs such as C22:5 and C22:6, and lower levels of oleate (C18:1Δ9) and C16 FAs, as compared to the plasma lipid FAs of the human host [31,45]. *Leishmania* spp. have high content of C18:2, C18:1 and C22 PUFAs, but also have α-linolenate (C18:3Δ9,12,15) and trace amounts of γ-linolenate (C18:3Δ6,9,12) [31,46]. 70–80% of the total FAs are represented by C16–18 species. Unsaturated FAs account for about 80% of them. The ratio of unsaturated/saturated FAs increases when decreasing the growth temperature, suggesting an important role of PUFA biosynthesis in the regulation of membrane fluidity [46–48].

Unsaturated FAs are synthesized in eukaryotes by an aerobic pathway [49]. This introduction of double bonds (desaturation) utilizes molecular oxygen and reducing equivalents obtained from an electron transport chain. The enzymes responsible, named desaturases, are integral membrane proteins of the endoplasmic

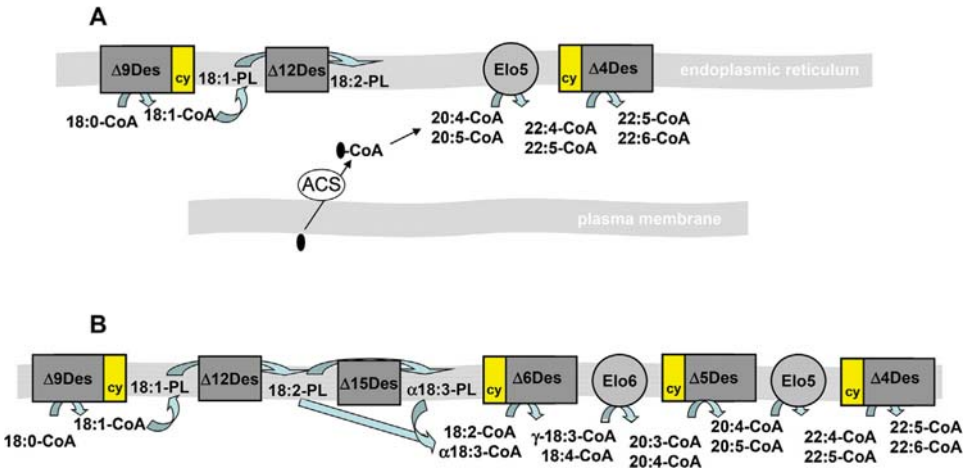

Fig. 3. Biosynthesis of unsaturated fatty acids. (A) Pathways found in *Trypanosoma* spp. (B) Pathway found in *Leishmania* spp. Δ*x*Des, desaturases that introduce a double bond between carbon *x* and *x* + 1 counting from the carboxyl end; ACS, acyl-CoA synthetase; cy, cytochrome-like domain; Elo5 and Elo6, KS isozymes of the ELO system which recognize PUFA substrates with the first double bond at carbons 5 and 6, respectively; PL, phospholipid.

reticulum. They can be classified as: Type I, which use saturated acyl-CoA substrates, usually introducing a double bond in the middle of the aliphatic chain; Type II or methyl-end desaturases, which introduce double bonds near the methyl end of unsaturated fatty acyl moieties of phospholipids and Type III or front-end desaturases, which introduce double bonds at the carboxylic end (front end) of polyunsaturated fatty acyl-CoAs. Front-end desaturases contain a cytochrome *b5*-like N-terminal domain. The active site of desaturases is supposed to be a di-iron center coordinated by eight histidine residues distributed along the protein, grouped in three characteristic boxes with the consensus motifs $HX_{3-4}H$, $HX_{2-3}HH$ and $HX_{2-3}HH$. An exception is found in Type III desaturases where the third box is QX_2HH. The glutamine and histidines are all essential for catalytic activity, with His boxes and the N- and C-terminus of desaturases facing the cytosol [49].

Evidence of desaturase activities in trypanosomatids has been documented previously. By using radioactive fatty acids such as stearic or oleic acid, different species have been shown to produce oleate, linoleate and linolenate [31,50]. A survey of the trypanosomatid genome projects showed numerous genes encoding putative proteins containing the structural features characteristic of FA desaturases. *L. major* LmjF.24.2250, *T. brucei* Tb927.8.6000 and orthologs found in *T. cruzi* (Table 1) and other *Leishmania* species, all showed significant similarities to Ole1p, the *S. cerevisiae* stearoyl-CoA Δ9 desaturase. They have a C-terminal cytochrome *b5*-like domain. Expression of LmjF.24.2250 in a *S. cerevisiae Ole1* mutant reverted its oleic acid auxotrophy [Uttaro, unpublished findings], converting up to the 85% of endogenous C18:0 into C18:1Δ9, but only 9% of C16:0 to C16:1Δ9. This confirmed the presence of Type I desaturases in trypanosomatids and this high specificity for stearate is in good agreement to the low amount of C16:1 found in these parasites (Fig. 3). LmjF.14.0510 is another putative enzyme with similarity to stearoyl-CoA desaturases, also containing a C-terminal cytochrome *b5* domain. It shares 44% identity with LmjF.24.2250 and has orthologs in other *Leishmania* species but not in trypanosomes. Stearoyl-CoA Δ9 desaturase was shown to be essential for trypanosomes, as knock down by RNAi in both procyclic and bloodstream forms of *T. brucei* drastically inhibited their growth in cultures [51]. Moreover, chemical inhibition by using Isoxyl (Thiocarlide) or 10-thiastearate, in *T. cruzi* epimastigotes [52] and in both stages of *T. brucei* [51], had similar effects on parasite growth. Interestingly, Isoxyl, a thiourea successfully used in the 1960s to treat tuberculosis and recently described as an inhibitor of *Mycobacterium tuberculosis* stearoyl-CoA desaturase [53], appeared to be trypanocidal in animal infections: its administration in both *T. cruzi* and *T. brucei* experimental infections reduced the parasitemia and increased the survival of infected mice [51,52].

T. brucei Tb927.2.3080 shares high similarity to fungal methyl-end desaturases. After expression in yeast we were able to characterize it as a Δ12 desaturase, which converts efficiently endogenous oleate to linoleate and to a lesser extent, palmitoleate (C16:1Δ9) to C16:2Δ9,12. In the yeast system the trypanosome Δ12 desaturase was able to receive electrons from the endogenous cytochrome *b5* and also from the cytochrome domain of Ole1, suggesting that they might form a multienzymatic complex in the endoplasmic reticulum. A similar interaction between two or more types of desaturases and electron donors may occur in trypanosomes. The activity was stimulated when expressed at low temperature. Supplementing the media with PUFAs had an opposite effect [54]. Both findings suggest that the enzyme is able to sense the membrane fluidity, resulting in modulation of its activity. It is the expected behavior of an enzyme probably involved in cold adaptation, allowing the parasites to cope with the drastic changes they experience when passing from a mammalian host to the insect vector and back. Orthologs of *T. cruzi* and *L. major* (Table 1) were functionally characterized, showing Δ12 desaturase activities as well. Interestingly, these enzymes have an opposite behavior compared to *T. brucei* desaturase being more active under conditions supposed to increase membrane fluidity [55]. It means that in these parasite species, Δ12 desaturases are probably not involved in cold adaptation. It has been shown that the GPI anchor of mucins in *T. cruzi* trypomastigotes, the non-proliferative, infective bloodstream form, contains C18:1 and C18:2 FAs. No unsaturated FAs were found in other GPI-anchored molecules of the metacyclic, trypomastigote or epimastigote forms [56]. GPI moieties are involved in triggering the host innate immunity, which is the initial line of defense against the invading parasite. These glycoconjugates also contribute to the development of acquired immunity by binding to Toll-like receptors (TLRs) on the surface of host macrophages, initiating a signaling cascade that culminates in the production of pro-inflammatory cytokines. GPI anchors of trypomastigote mucins activate TLR2 [57], having pro-inflammatory activity, whereas other glycoconjugates were inactive [56,58,59]. It suggests that unsaturated FAs, and thus desaturases, could be involved in modulating the infection and that inhibiting *T. cruzi* desaturases could render the parasite more susceptible to the host immune system's attack. We have designed thiastearic FA isomers in order to inhibit Δ12 desaturases specifically. These compounds were effective against *T. cruzi*

epimastigotes [52] and procyclic and bloodstream forms of *T. brucei* [60], inhibiting their growth with effective concentrations (EC_{50}) of 2–50 μM. Stearolic acid (9-octadecynoic acid) was also effective against *T. cruzi* epimastigotes and *T. brucei* bloodstream form cells, with EC_{50}s of 1 and 0.1 μM, respectively, by inhibiting specifically their Δ12 desaturases [61]. The knock down of the expression of this enzyme by RNAi drastically inhibited the growth of *T. brucei* procyclic and bloodstream stages, providing strong evidence for the essentiality of this enzyme [60]. Simultaneous inhibition of Δ12 desaturase and stearoyl-CoA desaturase has a notable synergistic effect in both trypanosome species. A combination of equimolar concentrations of Isoxyl and stearolic acid showed an EC_{50} of 5 nM [61]. Genetic or chemical ablation of Δ12 desaturase was trypanolytic, whereas ablation of stearoyl-CoA desaturase had a trypanostatic effect, resulting in an increased number of aberrant-looking bi-flagellate cells. Mammals lack methyl-end desaturases but have stearoyl-CoA Δ9 desaturases. It has been reported that Isoxyl or thiastearates did not affect growth of cultured mammalian cells and did not show toxicity against humans and mice. Moreover, we found that trypanosomal Δ9 desaturases can be selectively inhibited also by GS-456332, a compound designed to inhibit the human enzyme in order to treat dyslipidemias [61]. These findings show that both desaturases have potential as anti-parasite drug targets.

L. major has two additional genes (LmjF.10.0010 and 10.1320), encoding 100% identical putative methyl-end desaturases, which are located at both ends of chromosome 10. The encoded proteins share 75% identity with LmjF.33.3270 and were characterized as Δ15 desaturases with high specificity for linoleate that is converted to α-linolenate (C18:3Δ9,12,15), the main C18:3 isomer found in *Leishmania* spp. (Fig. 3) [55].

Synthesis of longer PUFAs requires the alternate action of front-end desaturases and the ELO system [49]. Both C18:2Δ9,12 and C18:3Δ9,12,15 are substrates of the front-end Δ6 desaturase found in *L. major* (LmjF.36.6950), which converts them to γ-linolenate (C18:3Δ6,9,12) and C18:4Δ6,9,12,15, respectively [62]. These PUFAs are now substrates of LmElo6 (LmjF.32.1160) which elongates them to C20:3Δ8,11,14 and C20:4Δ8,11,14,17, respectively. The sequential action of Δ5 desaturase (LmjF.07.1090) and LmElo5 (LmjF.05.1170) produces C22:4Δ7,10,13,16 and C22:5Δ7,10,13,16,19 which are finally converted to C22:5Δ4,7,10,13,16 and C22:6Δ4,7,10,13,16,19 by Δ4 desaturase (LmjF.14.1340) [42,62]. These final products and some of the intermediates, although representing minor components, can be detected not only in extracts of *L. major*, but also in *T. brucei* and *T. cruzi*. Notably, trypanosomes lack Δ6 and Δ5 desaturases and Elo6, but conserve functional Elo5 and Δ4 desaturases. This allows parasites to synthesize the same final products by using C20 PUFA substrates taken from the mammalian host (Fig. 3). Why these enzymes were conserved in *Trypanosoma* parasites and the functions of C22 PUFAs are unresolved issues. The availability of C20 substrates is expected to be sporadic for the insect stages, depending of the blood meal.

5. Sources of substrates for biosynthesis of fatty acids

The ELO system requires butyryl-CoA as primer and malonyl-CoA as two-carbon donor [40,41]. Additionally, other saturated or unsaturated acyl-CoAs can be elongated by the system. These acyl-CoAs are obtained by the cell by means of the scavenging mechanisms described in Section 2 or by recycling acyl moieties from triglycerides or membrane lipids. Malonyl-CoA is synthesized from acetyl-CoA by Acetyl-CoA Carboxylase (ACC). Tb927.8.7100 (Table 1) was functionally characterized as the *T. brucei* ACC, being active in both procyclic and bloodstream stages of the parasite.

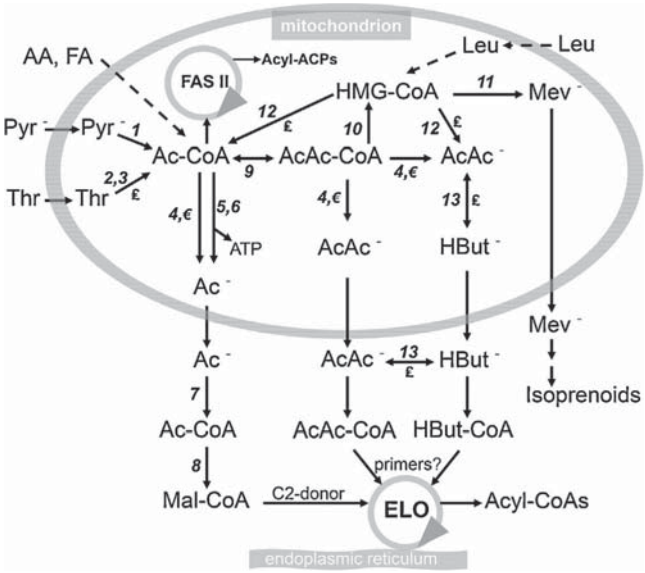

Fig. 4. Hypothetical pathways involved in producing substrates for *de novo* fatty acid biosynthesis in trypanosomatids. £, enzymes absent in *Leishmania* spp.; €, enzyme without ortholog in *T. cruzi*; AA, amino-acids (alanine, isoleucine, serine, threonine); Ac^-, acetate; $AcAc^-$, acetoacetate; FA, fatty acids; $HBut^-$, β-hydroxybutyrate; HMG^-, 3-hydroxy-3-methylglutarate; Leu, leucine; Mal^-, malonate; Mev^-, mevalonate; Pyr^-, pyruvate; Thr, threonine; Dashed arrows, pathways present in *Leishmania* spp. (1) pyruvate dehydrogenase complex; (2) threonine dehydrogenase; (3) 2-amino-3-ketobutyrate-CoA ligase; (4) acetyl-CoA thioesterase (ACH); (5) acetate:succinate CoA-transferase (ASCT); (6) succinyl-CoA synthetase; (7) acetyl-CoA synthetase; (8) acetyl-CoA carboxylase (ACC); (9) thiolase; (10) HMG-CoA synthetase; (11) HMG-CoA reductase; (12) HMG-CoA lyase; (13) $HBut^-$ dehydrogenase (HBDH) of unknown localization.

The enzyme is located in the cytosol (Fig. 4) [63] displaying a punctate pattern by immunofluorescence analysis, probably due to the polymeric nature of the protein, like that found for ACCs of vertebrates. The growth phenotype of ACC-ablated *T. brucei* was similar to that of the enoyl-CoA reductase RNAi cells (Section 3). Procyclic stages did not grow efficiently in lipid depleted media whereas bloodstream forms grew normally in standard or depleted media, although ACC RNAi resulted in an attenuated virulence in a mouse infection model.

Eukaryotes use a citrate/malate shuttle to transfer acetyl group equivalents from the mitochondrial matrix to the cytosol. Trypanosomatids lack this shuttle but translocate acetate generated in the mitochondrion to the cytosol where it is converted to acetyl-CoA by an AMP-forming acetyl-CoA synthetase [64]. Knocking down the synthetase (Tb927.8.2520) in the procyclic and bloodstream stages of *T. brucei* resulted in a drastic reduction of ^{14}C-incorporation from radiolabeled glucose or acetate into *de novo* synthesized FAs and the cells died, even when cultured in lipid rich media [64,65]. It strongly suggests that most if not all the acetyl-CoA used in FA biosynthesis is produced by this single synthetase. Exogenous acetate is not a physiological carbon source for *de novo* FA biosynthesis in trypanosomatids, and it is taken up very inefficiently by *T. brucei* bloodstream forms [40]. *T. brucei* uses glucose and threonine (preferentially in procyclic forms) as carbon sources for FA synthesis [65,66]. Glucose is converted to pyruvate during glycolysis, which enters the mitochondrion and is converted to acetyl-CoA by pyruvate dehydrogenase [2]. Threonine is converted to equimolar amounts of glycine and acetyl-CoA by the sequential action of threonine dehydrogenase (Tb927.6.2790) and 2-amino-3-ketobutyryl-CoA ligase (Tb927.8.6060). Both enzymes are located in the mitochondrion as well (Fig. 4) [65,66] and have orthologs in other trypanosome species (Table 1). *Leishmania* spp. do not have obvious orthologs of these threonine-degrading

enzymes, but nonetheless carbon atoms from threonine and substrates which are metabolized to acetyl-CoA, like glucose, palmitic acid, alanine, serine and isoleucine, all are readily incorporated into FAs [67]. Interestingly, acetyl-CoA is not a preferred precursor for the biosynthesis of sterols in *Leishmania* species. Instead, leucine provides the major carbon source for sterol production in cultured parasites [67,68]. In trypanosomes, the first steps of isoprenoid synthesis localizes in the mitochondrion were acetyl-CoA is converted to mevalonate by the sequential action of thiolase, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthetase and HMG-CoA reductase [69,70]. Acetyl-CoA may also be used in the FAS II system but most of it is converted into acetate by two different mitochondrial enzymes, acetyl-CoA thioesterase (ACH) and acetate:succinate CoA-transferase (ASCT), the latter involved in substrate-level ATP production in combination with succinyl-CoA synthetase [65,71–73]. This acetate is mainly excreted, but part is used in the cytosol by the acetyl-CoA synthetase.

The origin of the ELO primer is as yet unknown. Butyryl-CoA was a good substrate for the cell-free system described above (Section 3), but another metabolite could be the physiological primer. The likeliest candidates are β-hydroxybutyryl-CoA and acetoacetyl-CoA. Acetoacetate and β-hydroxybutyrate could be derived from the first cycle of the FAS II system, but this implies that ELO would be dependent on FAS II activity, which is not the case, as demonstrated by Englund and coworkers (Section 3). Alternatively, acetoacetate may be generated by the thiolase involved in isoprenoid metabolism. A thiolase with robust activity in both directions, the synthesis of acetoacetyl-CoA from acetyl-CoA and the reverse reaction, was characterized in procyclic stage of *T. brucei* [70,74]. In this case, both pathways, FA and sterol biosynthesis, would be linked by the thiolase (Fig. 4). Notably, *T. brucei* and *T. cruzi* genomes encode a putative HMG-CoA lyase (Tb927.4.2700, TcCLB.506635.80, Table 1), an enzyme that catalyses the conversion of HMG-CoA to acetyl-CoA and acetoacetate. It could be speculated that the balance between HMG-CoA synthetase and lyase activities would modulate the carbon flow through each pathway, with both enzymes probably being highly regulated. It has been described that procyclic trypanosomes produce and excrete β-hydroxybutyrate when growing on proline as energy source [75] or growing on glucose under conditions that promote acetyl-CoA accumulation, like ablation of ASCT (72) or phosphoenolpyruvate carboxykinase [76]. Part of acetyl-CoA could be converted to acetoacetyl-CoA and then to β-hydroxybutyrate by the sequential action of ACH and β-hydroxybutyrate dehydrogenase (HBDH, Fig. 4). Bacterial like HBDHs were predicted to be present in *T. brucei* and *T. cruzi* (TriTrypDB, Table 1) and biochemically characterized in *T. brucei* [77]. ACH showed highest activity using acetyl-CoA as substrate but also with butyryl-CoA, acetoacetyl-CoA and β-hydroxybutyryl-CoA [73]. It makes possible that a pathway involving thiolase, ACH and HBDH would be responsible of β-hydroxybutyrate synthesis which, once in the cytosol, could be converted back to the CoA thioester to prime the ELO system (Fig. 4). Although there is no obvious ACH ortholog in *T. cruzi*, the existence of alternative ways to produce acetoacetate and β-hydroxybutyrate from the corresponding CoA thioesters in this species should not be ruled out. The presence of a mitochondrial HMG-CoA synthetase in *L. major*, in addition to the leucine pathway to synthesize mevalonate [69] and the lack of clear HMG-CoA lyase and HBDH orthologs, make it difficult to realize whether *Leishmania* spp. could modulate FA and isoprenoid pathways in a similar way as that proposed here for *Trypanosoma* spp.

6. Concluding remarks

The conditions encountered by these flagellates living as parasites provided, most probably, the evolutionary pressure that conducted to the peculiar strategies for FA acquisition described here. It makes relevant to further explore these specific pathways in order to validate the processes, and the enzymes involved, as targets for the development of new therapeutic drugs. However, some metabolic routes did not show the relevance we expected in that respect. This is the case for the ELO system or the synthesis of cytoplasmic malonyl-CoA by ACC, which are apparently not essential when trypanosomatids proliferate in lipid rich environments like the host bloodstream. In contrast, the mitochondrial FAS II system was shown to be essential, even though it is responsible for only 10% of the total FAs synthesized by the cell. The inhibition of FAS II has multiple consequences, resulting in aberrant mitochondria and loss of cell viability. These findings suggest to us that, contrary to what one might suppose, the salvage mechanisms described in Section 2 could supply enough saturated FAs and part of unsaturated FAs that the trypanosomes require. In this instance, *de novo* FA synthesis could be essential only for establishing long-term infections in mammals and to reach a high parasitemia. On the other hand, *in situ* desaturation appears to be essential, probably due to the role of unsaturated FAs in maintaining necessary membrane fluidity and in modulating the host immune system. Cytoplasmic synthesis of acetyl-CoA is also essential, although it is not necessarily related to merely the synthesis of FAs, but also other biosynthetic pathways or in regulating the expression of genes by epigenetic mechanisms, such as chromatin acetylation.

Acknowledgements

The author thanks Dr. Paul A.M. Michels for helpful comments and suggestions on the manuscript. The author gratefully acknowledges the TriTryps genome projects consortium, The Institute of Genomic Research (TIGR) and The Sanger Institute for the availability of sequence data. A.D.U. is member of Carrera del Investigador Científico, CONICET, Argentina and received funds from the Fondo Nacional de Ciencia y Tecnología, Ministerio de Ciencia, Tecnología e Innovación Productiva de la Nación, Argentina, by grants PICT 2007 N° 654 and PICT 2012 N° 1301.

References

[1] Barrett MP, Burchmore RJS, Stich A, Lazzari JO, Frasch AC, Cazzulo JJ, et al. The trypanosomiases. Lancet 2003;362:469–80.
[2] Gualdrón-López M, Brennand A, Hannaert V, Quiñones W, Cáceres AJ, Bringaud F, et al. When, how and why glycolysis became compartmentalised in the Kinetoplastea. A new look at an ancient organelle. Int J Parasitol 2012;42:1–20.
[3] Ginger ML. Trypanosomatid biology and euglenozoan evolution: new insights and shifting paradigms revealed through genome sequencing. Protist 2005;156:377–92.
[4] Roberts CW, McLeod R, Rice DW, Ginger M, Chance ML, Goad LJ. Fatty acid and sterol metabolism: potential antimicrobial targets in apicomplexan and trypanosomatid parasitic protozoa. Mol Biochem Parasitol 2003;126:129–42.
[5] Zhang K, Beverley SM. Phospholipid and sphingolipid metabolism in *Leishmania*. Mol Biochem Parasitol 2010;170:55–64.
[6] Zhang K, Bangs JD, Beverley SM. Sphingolipids in parasitic protozoa. Adv Exp Med Biol 2010;688:238–48.
[7] Smith TK, Bütikofer P. Lipid metabolism in *Trypanosoma brucei*. Mol Biochem Parasitol 2010;172:66–79.
[8] Ramakrishnan S, Serricchio M, Striepen B, Bütikofer P. Lipid synthesis in protozoan parasites: a comparison between kinetoplastids and apicomplexans. Prog Lipid Res 2013;52:488–512.
[9] van Hellemond JJ, Tielens AG. Adaptations in the lipid metabolism of the protozoan parasite *Trypanosoma brucei*. FEBS Lett 2006;580:5552–8.
[10] Lee SH, Stephens JL, Englund PT. A fatty-acid synthesis mechanism specialized for parasitism. Nat Rev Microbiol 2007;5:287–97.
[11] Coppens I, Courtoy PJ. The adaptative mechanisms of *Trypanosoma brucei* for sterol homeostasis in its different life-cycle environments. Annu Rev Microbiol 2000;54:129–56.
[12] Soares MJ, de Souza W. Endocytosis of gold-labeled proteins and LDL by *Trypanosoma cruzi*. Parasitol Res 1991;77:461–8.
[13] Andrade-Neto VV, Cicco NN, Cunha-Junior EF, Canto-Cavalheiro MM, Atella GC, Torres-Santos EC. The pharmacological inhibition of sterol biosynthesis in *Leishmania* is counteracted by enhancement of LDL endocytosis. Acta Trop 2011;119:194–8.

[14] Coppens I, Levade T, Courtoy PJ. Host plasma low density lipoprotein particles as an essential source of lipids for the bloodstream forms of *Trypanosoma brucei*. J Biol Chem 1995;270:5736–41.
[15] De Cicco NN, Pereira MG, Corrêa JR, Andrade-Neto VV, Saraiva FB, Chagas-Lima AC, et al. LDL uptake by *Leishmania amazonensis*: involvement of membrane lipid microdomains. Exp Parasitol 2012;130:330–40.
[16] Gillett MP, Owen JS. Characteristics of the binding of human and bovine high-density lipoproteins by bloodstream forms of the African trypanosome, *Trypanosoma brucei brucei*. Biochim Biophys Acta 1992;1123:239–48.
[17] Vanhollebeke B, Pays E. The trypanolytic factor of human serum: many ways to enter the parasite, a single way to kill. Mol Microbiol 2010;76:806–14.
[18] Stephens NA, Kieft R, Macleod A, Hajduk SL. Trypanosome resistance to human innate immunity: targeting Achilles' heel. Trends Parasitol 2012;28:539–45.
[19] Uzureau P, Uzureau S, Lecordier L, Fontaine F, Tebabi P, Homblé F, et al. Mechanism of *Trypanosoma brucei gambiense* resistance to human serum. Nature 2013;501:430–4.
[20] Rauch AL, Buckalew Jr VM. Plasma volume expansion increases lysophosphatidylcholine and digitalis-like activity in rat plasma. Life Sci 1988;42:1189–97.
[21] Bowes AE, Samad AH, Jiang P, Weaver B, Mellors A. The acquisition of lysophosphatidylcholine by African trypanosomes. J Biol Chem 1993;268:13885–92.
[22] Glatz JF, Luiken JJ, Bonen A. Membrane fatty acid transporters as regulators of lipid metabolism: implications for metabolic disease. Physiol Rev 2010;90:367–417.
[23] Voorheis HP. Fatty acid uptake by bloodstream forms of *Trypanosoma brucei* and other species of the kinetoplastida. Mol Biochem Parasitol 1980;1:177–86.
[24] Berman JD, Gallalee JV, Best JM, Hill T. Uptake, distribution, and oxidation of fatty acids by *Leishmania mexicana* amastigotes. J Parasitol 1987;73:555–60.
[25] Black PN, DiRusso CC. Yeast acyl-CoA synthetases at the crossroads of fatty acid metabolism and regulation. Biochim Biophys Acta 2007;1771:286–98.
[26] Jiang DW, Englund PT. Four *Trypanosoma brucei* fatty acyl-CoA synthetases: fatty acid specificity of the recombinant proteins. Biochem J 2001;358:757–61.
[27] Milne KG, Ferguson MA. Cloning expression, and characterization of the acyl-CoA-binding protein in African trypanosomes. J Biol Chem 2000;275:12503–8.
[28] Milne KG, Güther ML, Ferguson MA. Acyl-CoA binding protein is essential in bloodstream form *Trypanosoma brucei*. Mol Biochem Parasitol 2001;112:301–4.
[29] Vance DE, Vance JE. Biochemistry of lipids lipoproteins and membranes. 4th ed. Amsterdam: Elsevier; 2002.
[30] Leonard AE, Pereira SL, Sprecher H, Huang Y-S. Elongation of long-chain fatty acids. Prog Lipid Res 2004;43:36–54.
[31] Haughan PA, Goad LJ. Lipid biochemistry of trypanosomatids. In: Coombs GH, North MJ, editors. Biochemical protozoology. London, Washington, DC: Taylor and Frances; 1991. p. 312–28.
[32] Dixon H, Ginger CD, Williamson J. The lipid metabolism of blood and culture forms of *Trypanosoma lewisi* and *Trypanosoma rhodesiense*. Comp Biochem Physiol 1971;39B:247–66.
[33] Stephens JL, Lee SH, Paul KS, Englund PT. Mitochondrial fatty acid synthesis in *Trypanosoma brucei*. J Biol Chem 2007;282:4427–36.
[34] Autio J, Guler JL, Kastaniotis AJ, Englund PT, Hiltunen JT. The 3-hydroxyacyl-ACP dehydratase of mitochondrial fatty acid synthesis in *Trypanosoma brucei*. FEBS Lett 2008;582:729–33.
[35] Gurvitz A. Identification of the *Leishmania major* proteins LmjF07.0430, LmjF07.0440 and LmjF27.2440 as components of fatty acid synthase II. J Biomed Biotechnol 2009;2009:950864.
[36] Spalding MD, Prigge ST. Lipoic acid metabolism in microbial pathogens. Microbiol Mol Biol Rev 2010;74:200–28.
[37] Guler JL, Kriegova E, Smith TK, Lukes J, Englund PT. Mitochondrial fatty acid synthesis is required for normal mitochondrial morphology and function in *Trypanosoma brucei*. Mol Microbiol 2008;67:1125–42.
[38] Clayton AM, Guler JL, Povelones ML, Gluenz E, Gull K, Smith TK, et al. Depletion of mitochondrial acyl carrier protein in bloodstream-form *Trypanosoma brucei* causes a kinetoplast segregation defect. Eukaryotic Cell 2011;10:286–92.
[39] Paul KS, Jiang D, Morita YS, Englund PT. Fatty acid synthesis in African trypanosomes: a solution to the myristate mystery. Trends Parasitol 2001;17:381–7.
[40] Morita YS, Paul KS, Englund PT. Specialized fatty acid synthesis in African trypanosomes: myristate for GPI anchors. Science 2000;288:140–3.
[41] Lee SH, Stephens JL, Paul KS, Englund PT. Fatty acid synthesis by elongases in trypanosomes. Cell 2006;126:691–9.
[42] Livore VI, Tripodi KE, Uttaro AD. Elongation of polyunsaturated fatty acids in trypanosomatids. FEBS J 2007;274:264–74.
[43] Paul S, Gable K, Beaudoin F, Cahoon E, Jaworski J, Napier JA, et al. Members of the arabidopsis FAE1-like 3-ketoacyl-CoA synthase gene family substitute for the Elop proteins of *Saccharomyces cerevisiae*. J Biol Chem 2006;281:9018–29.
[44] Opperdoes FR, Szikora JP. In silico prediction of the glycosomal enzymes of *Leishmania major* and trypanosomes. Mol Biochem Parasitol 2006;147:193–206.
[45] Mellors A, Samad A. The acquisition of lipids by African trypanosomes. Parasitol Today 1989;5:239–44.
[46] Glew RH, Saha AK, Das S, Remaley AT. Biochemistry of the *Leishmania* species. Microbiol Rev 1988;52:412–32.
[47] Florin-Christensen M, Florin-Christensen J, de Isola ED, Lammel E, Meinardi E, Brenner RR, et al. Temperature acclimation of *Trypanosoma cruzi* epimastigote and metacyclic trypomastigote lipids. Mol Biochem Parasitol 1997;88:25–33.
[48] Sinensky M. Homeoviscous adaptation—a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. Proc Natl Acad Sci USA 1974;71:522–5.
[49] Uttaro AD. Biosynthesis of polyunsaturated fatty acids in lower eukaryotes. IUBMB Life 2006;58:563–71.
[50] de Lema MG, Aeberhard EE. Desaturation of fatty acids in *Trypanosoma cruzi*. Lipids 1986;21:718–20.
[51] Alloatti A, Gupta S, Gualdrón-López M, Nguewa PA, Altabe SG, Deumer G, et al. Stearoyl-CoA desaturase is an essential enzyme for the parasitic protist *Trypanosoma brucei*. Biochem Biophys Res Commun 2011;412:286–90.
[52] Alloatti A, Testero SA, Uttaro AD. Chemical evaluation of fatty acid desaturases as drug targets in *Trypanosoma cruzi*. Int J Parasitol 2009;39:985–93.
[53] Phetsuksiri B, Jackson M, Scherman H, McNeil M, Besra GS, Baulard AR, et al. Unique mechanism of action of the thiourea drug Isoxyl on *Mycobacterium tuberculosis*. J Biol Chem 2003;278:53123–30.
[54] Petrini GA, Altabe SG, Uttaro AD. *Trypanosoma brucei* oleate desaturase may use a cytochrome b5-like domain in another desaturase as an electron donor. Eur J Biochem 2004;271:1079–86.
[55] Alloatti A, Uttaro AD. Highly specific methyl-end fatty-acid desaturases of trypanosomatids. Mol Biochem Parasitol 2011;175:126–32.
[56] Almeida IC, Gazzinelli RT. Proinflammatory activity of glycosylphosphatidylinositol anchors derived from *Trypanosoma cruzi*: structural and functional analyses. J Leukoc Biol 2001;70:467–77.
[57] Campos MA, Almeida IC, Takeuchi O, Akira S, Valente EP, Procópio DO, et al. Activation of Toll-like receptor-2 by glycosylphosphatidylinositol anchors from a protozoan parasite. J Immunol 2001;167:416–23.
[58] Oliveira AC, Peixoto JR, de Arruba LB, Campos MA, Gazzinelli RT, Golenbock DT, et al. Expression of functional TLR4 confers proinflammatory responsiveness to *Trypanosoma cruzi* glycoinositolphospholipids and higher resistance to infection with *T. cruzi*. J Immunol 2004;173:5688–96.
[59] Gazzinelli RT, Denkers EY. Protozoan encounters with Toll-like receptor signalling pathways: implications for host parasitism. Nat Rev Immunol 2006;6:895–906.
[60] Alloatti A, Gupta S, Gualdrón-López M, Igoillo-Esteve M, Nguewa PA, Deumer G, et al. Genetic and chemical evaluation of *Trypanosoma brucei* oleate desaturase as a candidate drug target. PLoS ONE 2010;5:e14239.
[61] Alloatti A, Tripodi KE, Uttaro AD. Synergistic effect of inhibitors of fatty acid desaturases on *Trypanosoma* parasites. Parasitol Res 2013;112:3289–94.
[62] Tripodi KE, Buttigliero LV, Altabe SG, Uttaro AD. Functional characterization of front-end desaturases from trypanosomatids depicts the first polyunsaturated fatty acid biosynthetic pathway from a parasitic protozoan. FEBS J 2006;273:271–80.
[63] Vigueira PA, Paul KS. Requirement for acetyl-CoA carboxylase in *Trypanosoma brucei* is dependent upon the growth environment. Mol Microbiol 2011;80:117–32.
[64] Rivière L, Moreau P, Allmann S, Hahn M, Biran M, Plazolles N, et al. Acetate produced in the mitochondrion is the essential precursor for lipid biosynthesis in procyclic trypanosomes. Proc Natl Acad Sci USA 2009;106:12694–9.
[65] Mazet M, Morand P, Biran M, Bouyssou G, Courtois P, Daulouède S, et al., Franconi JM, Moreau P. Revisiting the central metabolism of the bloodstream forms of *Trypanosoma brucei*: production of acetate in the mitochondrion is essential for parasite viability. PLoS Neglect Trop Dis 2013;7:e2587.
[66] Millerioux Y, Ebikeme C, Biran M, Morand P, Bouyssou G, Vincent IM, et al. The threonine degradation pathway of the *Trypanosoma brucei* procyclic form: the main carbon source for lipid biosynthesis is under metabolic control. Mol Microbiol 2013;90:114–29.
[67] Ginger ML, Chance ML, Goad LJ. Elucidation of carbon sources used for the biosynthesis of fatty acids and sterols in the trypanosomatid *Leishmania mexicana*. Biochem J 1999;342:397–405.
[68] Ginger ML, Chance ML, Sadler IH, Goad LJ. The biosynthetic incorporation of the intact leucine skeleton into sterol by the trypanosomatid *Leishmania mexicana*. J Biol Chem 2001;276:11674–82.
[69] Carrero-Lérida J, Pérez-Moreno G, Castillo-Acosta VM, Ruiz-Pérez LM, González-Pacanowska D. Intracellular location of the early steps of the isoprenoid biosynthetic pathway in the trypanosomatids *Leishmania major* and *Trypanosoma brucei*. Int J Parasitol 2009;39:307–14.
[70] Mazet M, Harijan RK, Kiema TR, Haapalainen AM, Morand P, Morales J, et al. The characterization and evolutionary relationships of a trypanosomal thiolase. Int J Parasitol 2011;41:1273–83.
[71] Van Hellemond JJ, Opperdoes FR, Tielens AG. Trypanosomatidae produce acetate via a mitochondrial acetate:succinate CoA transferase. Proc Natl Acad Sci USA 1998;95:3036–41.
[72] Rivière L, van Weelden SW, Glass P, Vegh P, Coustou V, Biran M, et al. Acetyl:succinate CoA-transferase in procyclic *Trypanosoma brucei* gene identification and role in carbohydrate metabolism. J Biol Chem 2004;279:45337–46.
[73] Millerioux Y, Morand P, Biran M, Mazet M, Moreau P, Wargnies M, et al. ATP synthesis-coupled and -uncoupled acetate production from acetyl-CoA by mitochondrial acetate:succinate CoA-transferase and acetyl-CoA thioesterase in *Trypanosoma*. J Biol Chem 2012;287:17186–97.
[74] Harijan RK, Kiema TR, Karjalainen MP, Janardan N, Murthy MR, Weiss MS, et al. Crystal structures of SCP2-thiolases of Trypanosomatidae, human pathogens causing widespread tropical diseases: the importance for catalysis of the cysteine of the unique HDCF loop. Biochem J 2013;455:119–30.

[75] Coustou V, Biran M, Breton M, Guegan F, Rivière L, Plazolles N, et al. Glucose-induced remodeling of intermediary and energy metabolism in procyclic *Trypanosoma brucei*. J Biol Chem 2008;283:16342–54.
[76] Ebikeme C, Hubert J, Biran M, Gouspillou G, Morand P, Plazolles N, et al. Ablation of succinate production from glucose metabolism in the procyclic trypanosomes induces metabolic switches to the glycerol 3-phosphate/dihydroxyacetone phosphate shuttle and to proline metabolism. J Biol Chem 2010;285:32312–24.
[77] Shah TD, Hickey MC, Capasso KE, Palenchar JB. The characterization of a unique *Trypanosoma brucei* β-hydroxybutyrate dehydrogenase. Mol Biochem Parasitol 2011;179:100–6.