Biochimica et Biophysica Acta 1860 (2016) 363–370

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Iron-associated biology of *Trypanosoma brucei*

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ARTICLE INFO

Article history:
Received 31 July 2015
Received in revised form 22 October 2015
Accepted 29 October 2015
Available online 30 October 2015

Keywords:
Iron
Fe/S cluster
Heme
Trypanosoma
TAO

ABSTRACT

Background: Every eukaryote requires iron, which is also true for the parasitic protist *Trypanosoma brucei*, the causative agent of sleeping sickness in humans and nagana in cattle. *T. brucei* undergoes a complex life cycle during which its single mitochondrion is subject to major metabolic and morphological changes.
Scope of review: This review covers what is known about processes associated with iron–sulfur clusters and heme metabolism in *T. brucei*. We discuss strategies by which iron and heme are acquired and utilized by this model parasite, emphasizing the differences between its two life cycle stages residing in the bloodstream of the mammalian host and gut of the insect vector. Finally, the role of iron in the host–parasite interactions is discussed along with their possible exploitation in fighting these deadly parasites.
Major conclusions: The processes associated with acquisition and utilization of iron, distinct in the two life stages of *T. brucei*, are fine tuned for the dramatically different host environment occupied by them. Although the composition and compartmentalization of the iron–sulfur cluster assembly seem to be conserved, some unique features of the iron acquisition strategies may be exploited for medical interventions against these parasites.
General significance: As early-branching protists, trypanosomes and related flagellates are known to harbor an array of unique features, with the acquisition of iron being another peculiarity. Thanks to intense research within the last decade, understanding of iron–sulfur cluster assembly and iron metabolism in *T. brucei* is among the most advanced of all eukaryotes.

1. Introduction

Iron is a fundamental element for every living organism, indispensable for a number of cellular processes, such as synthesis of DNA, RNA and proteins, proliferation, respiration and regulation of gene expression [1,2]. The ability of iron to undergo a cyclic transformation between oxidized and reduced forms is its principal feature. However, due to the capacity of iron to take part in the Fenton reaction, which generates free-radicals prone to cause cellular damage, it is potentially toxic for any cell [3].

The availability of iron also plays an important role in the relationship between the pathogen and the host [4] with the kinetoplastid parasitic protist *Trypanosoma brucei* being no exception. *T. brucei* is subdivided into at least four sub-species: *T. b. brucei*, *T. b. evansi*, *T. b. gambiense*, and *T. b. rhodesiense* [5]. The latter two species are causative agents of the human West African and East African sleeping sickness, respectively [6]. *T. b. evansi* is a "petite" mutant of *T. brucei*, responsible for a disease called surra mostly in ruminants [7], while *T. b. brucei* causes the animal African trypanosomiasis (nagana) in cattle and some other mammals [5]. Being non-pathogenic to humans, as it is efficiently lysed by human serum, *T. b. brucei* is amenable to forward and reverse genetics, and thus most data covered by this review has been obtained from studies of this model flagellate.

T. brucei undergoes a complex digenetic life cycle, switching between a mammalian host (human, cattle, antelopes *etc.*) and an insect vector (tse-tse fly *Glossina* spp.). In response to radical differences of the host environment, the parasite undergoes substantial morphological and metabolic variations. The main developmental stages, also well tractable under laboratory culture conditions, are the mammalian-specific bloodstream stage (BS) and the insect-specific procyclic stage (PS). The latter stage carries a large reticulated mitochondrion with a regular ATP-producing respiratory chain, which is an adaptation of the flagellates to live in carbohydrate-poor conditions of its insect host [8,9]. In contrast, by utilizing glucose abundantly present in the mammalian bloodstream, the BS trypanosomes down-regulate their mitochondrion and in terms of energy production rely on glycolysis largely confined to specialized peroxisomes called glycosomes [10,11]. Due to these dramatic metabolic and environmental differences, the processes associated with iron-acquisition and utilization are rather dissimilar in the BS and PS of *T. brucei* and will therefore be discussed separately. Moreover, we review recent advances in our understanding

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http://dx.doi.org/10.1016/j.bbagen.2015.10.027
0304-4165/

of iron–sulfur (Fe/S) cluster protein biosynthesis and heme acquisition and utilization.

2. Iron acquisition

Pathogens acquire iron from their hosts *via* various sophisticated mechanisms suited to salvage it even at very low levels. The BS of *T. brucei* requires 40,000 iron atoms per cell, which have to be stored safely [12]. It is particularly relevant for this stage, since it lacks cytochromes and contains just a few iron-dependent enzymes, namely aconitase, alternative terminal oxidase, ribonucleotide reductase and superoxide dismutase [13]. Therefore, a tight regulation is needed for both scavenging and intracellular storage of iron. The BS trypanosomes acquire iron *via* the host's transferrin through a receptor-mediated endocytosis within the trypanosomal flagellar pocket [14], an invagination of the uninterrupted plasma membrane where the flagellum protrudes out [15] (Fig. 1A). When compared to its host's counterpart, the unique BS-specific transferrin receptor (TfR) is structurally diverse. It is encoded by two expression site-associated genes named ESAG6 and ESAG7, the alliance of which is required for transferrin binding [12,14,16]. ESAG6 possesses a glycosylphosphatidylinositol (GPI) anchor, which is responsible for localization of the receptor in the flagellar pocket [17]. ESAGs are co-transcribed along with the genes encoding variable surface glycoproteins (VSG), which constitute the surface coat of BS trypanosomes [18]. The host environment regulates the expression of TfR, with the amount of iron in the blood being the key factor. It was experimentally determined that the upregulated transcription of the TfR gene leads to its miss-localization in the plasma membrane besides the flagellar pocket membrane, most likely boosting interaction with more iron-carrying transferrin molecules. It is plausible that the capacity of trypanosomes to regulate expression of TfR reflects their ability to sense the diminishing availability of iron in the cytosol [16].

Endocytosis of the transferrin-receptor complex is followed by acidification in the late-endosome/lysosome, which releases the iron (Fig. 1A) [19]. The TfR protein is further proteolytically cleaved and cycled back to the flagellar pocket membrane. The released iron becomes available in the cytosol upon its transport *via* a mucolipin-like protein (MLP), localized in the endolysosomal system [20]. It has been suggested that iron is transported from the endolysosomal system into the cytosol through ferric reductase together with five putative divalent metal transporters containing a ZIP domain [21].

In the related human parasite *Leishmania amazonensis*, the plasma membrane ferric reductase (LFR1) reduces extracellular ferric iron to ferrous iron, which is subsequently transported into the cells by the ZIP family *Leishmania* iron transporter 1 (LIT1). This mechanism of

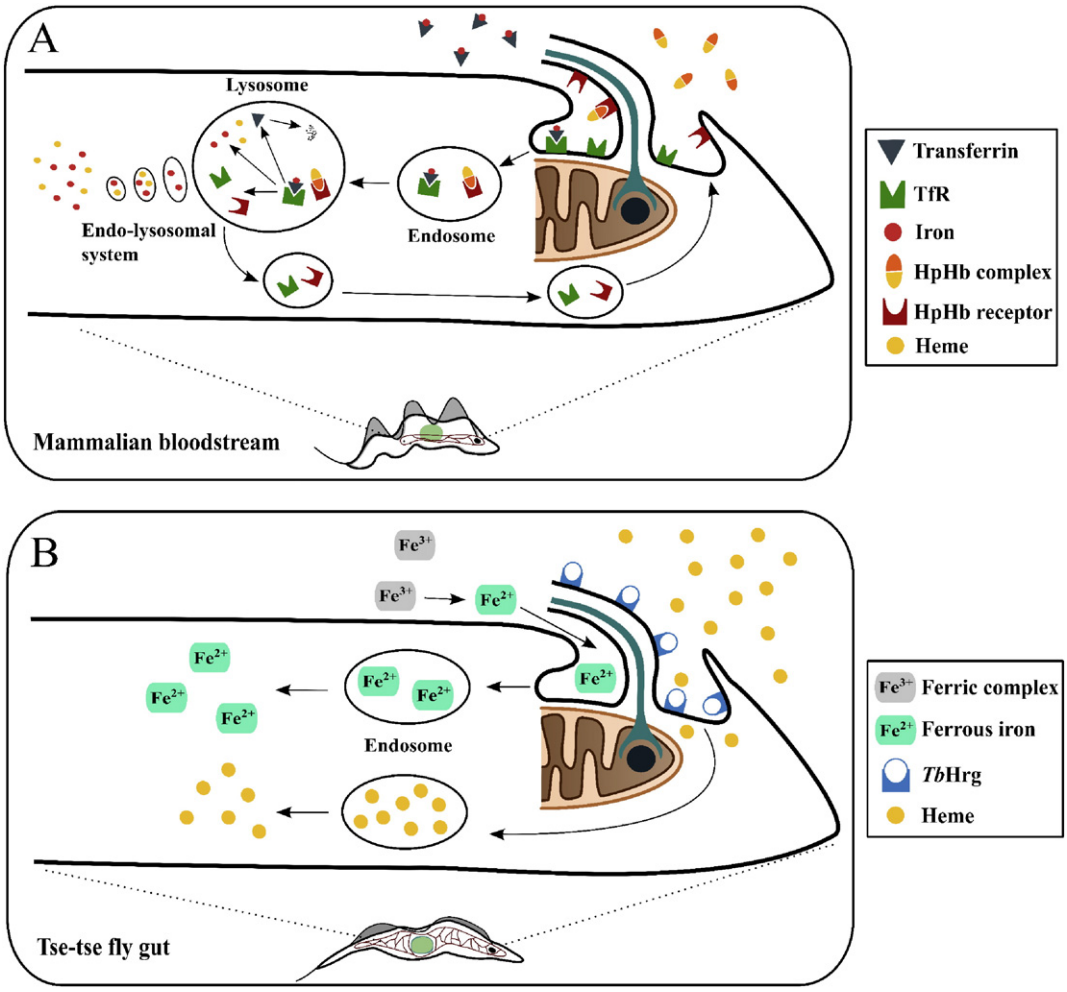

Fig. 1. Iron and heme acquisition in *Trypanosoma brucei*. A. In the mammalian bloodstream: *Iron acquisition*: In this transferrin-rich environment, the host transferrin forms a complex with transferrin receptor (TfR) located in the flagellar pocket. The complex is internalized by means of endocytosis. Subsequently, within the lysosome, the complex is cleaved and iron is released from the host transferrin upon proteolysis. Next iron is transported to the cytosol *via* the endo-lysosomal system and the TfR is cycled back to the flagellar pocket. *Heme acquisition*: The host haptoglobin–hemoglobin (Hp–Hb) complex is the source of heme for the BS cells. This complex is internalized by the haptoglobin–hemoglobin receptor (HpHbR-mediated) endocytosis. B. In the tse-tse fly gut: *Iron acquisition*: The insect vector gut is devoid of transferrin; hence, the PS flagellates acquire iron by a reductive mechanism. Available ferric complexes are reduced and subsequently endocytosed via the flagellar pocket. *Heme acquisition*: The insect vector gut lacks HpHbR; instead, the PS flagellates acquire heme *via* flagellar pocket using the *Tb*Hrg protein.

iron uptake is likely to be active under iron-deprived condition, since LFR1 and LIT1 do not seem to be essential when iron is in excess [22].

A recent RNA-seq analysis of the BS stage isolated from murine blood revealed a 200 fold amplified expression of TfR encoding ESAG6 and ESAG7 when compared to the tsetse fly salivary gland-sequestered flagellates [23]. This verifies the requirement of TfR to scavenge iron for the BS trypanosomes in mammalian blood. Unexpectedly, a comparative phylogenetic analysis of the surface proteins of trypanosomatids revealed that the basal-branching *Trypanosoma vivax* lacks the TfR gene, which is compatible with a rather late development of the gene following the emergence of the *T. congolense* and *T. brucei* clade [24].

Apart from transferrin, trypanosomes can take advantage of the iron-rich lactoferrin of the host, which is an iron-binding glycoprotein belonging to the transferrin family [25]. Indeed, it was demonstrated that *T. brucei* efficiently binds bovine lactoferrin, resulting in interaction of this host-derived protein with trypanosomal cytosolic glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [25]. In a similar fashion, mammalian GAPDH that is localized on the surface of macrophages binds both transferrin and lactoferrin [26,27]. Likewise, several other glycolytic proteins have been found on the surface of parasitic protists and helminths performing supplementary tasks on top of their usual intracellular functions. GAPDH has been reported on the surface of *Trichomonas vaginalis* and *Schistosoma japonicum* acting as a ligand of host-derived components. On the other hand, enolase helps *Plasmodium* spp. to interact with the epithelium of mosquito midgut [28,29].

When compared with the BS, we know less about the mechanisms by which the tsetse fly-dwelling PS acquire iron. Nevertheless, it has been shown recently that this is happening by a reductive mechanism from ferric complexes [30] (Fig. 1B). This process engages reduction of ferric (Fe^{3+}) to ferrous iron (Fe^{2+}) and its subsequent transport, enabling the PS flagellates to uptake iron from various ferric complexes in the transferrin-lacking insect gut [30] (Fig. 1B).

There is an example by which the host transferrin could be degraded by a specific trypanosomal cysteine protease, *Tb*CatB. Several studies showed that cysteine protease activity is essential for the survival of *T. brucei* [31–33], and even moderate ablation of *Tb*CatB was sufficient to generate a detrimental effect on the trypanosomes [34]. It was proposed that the role of *Tb*CatB in the degradation of host proteins including transferrin, is responsible for its high essentiality [34]. Indeed, these features qualify *Tb*CatB as a potential drug target.

In higher eukaryotes, biologically accessible forms of iron are used for Fe/S cluster biogenesis and heme synthesis in the mitochondria, into which free iron is imported in its reduced Fe^{2+} form. In *Saccharomyces cerevisiae* the inner mitochondrial membrane proteins Mrs3 and Mrs4, together with the membrane potential, are essential for this step [35]. In *T. brucei* the putative mitochondrial carrier protein *Tb*MCP17 (48% similar to yeast Mrs3 and human MFRN ion carriers), situated at the inner mitochondrial membrane, is possibly functioning as the iron importer into the organelle [36] (Fig. 2A).

3. Iron-containing enzyme cofactors

The biological relevance of iron lies in its role as an enzyme cofactor, principally in the electron-transfer chain and a broad range of metabolic activities. The iron-containing cofactors could be classified into three classes: Fe/S clusters, heme, and di-iron/mononuclear iron [37]. We will initially discuss the synthesis of Fe/S clusters in *T. brucei*, followed by the description of the intriguing heme-scavenging system and its subsequent exploitation, and finally the non-heme and non-Fe/S iron in this model protist.

3.1. Iron–sulfur clusters

Life likely originated in an 'iron–sulfur world' catalyzed by inorganic structures called Fe/S clusters [38,39]. In the contemporary world, Fe/S clusters function as versatile protein cofactors. Despite their simple chemical structures, their biosynthesis and subsequent incorporation into numerous target proteins are surprisingly complex. Being incorporated into DNA polymerases and helicases and the ribosomal biogenesis protein Rli1, to name just a few proteins, Fe/S clusters are clearly fundamental components of all extant cells [35,40].

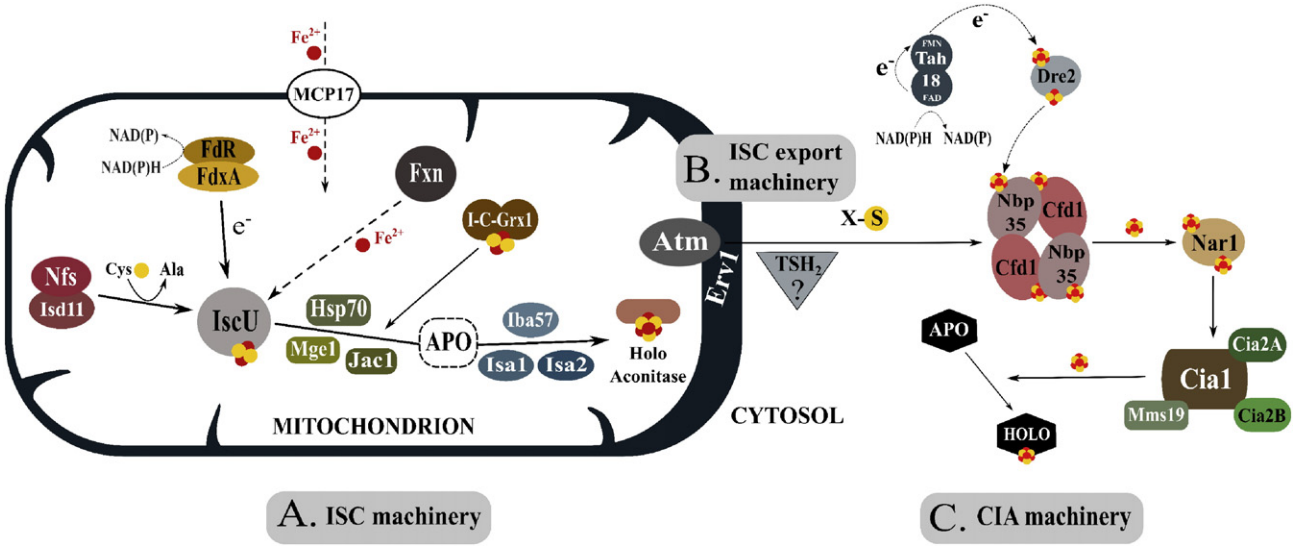

Fig. 2. Fe/S cluster protein biosynthesis in *T. brucei*. A. ISC machinery: For both mitochondrial and cytosolic Fe/S cluster protein biogenesis, the Nfs-Isd11-IscU module and subsequently ferredoxin (FdxA) are required. Ferrous iron (Fe^{2+}, red sphere) is imported by the putative MCP17 (putative function: broken arrow) into the mitochondrion. Frataxin (Fxn) is the subsequent ISC component, which may or may not be involved in iron storage (unclear function: broken arrow). The ultimate step requires the mitochondrial Hsp70, Mge1 and Jac1, guaranteeing incorporation of Fe/S clusters into several apo-proteins. The mitochondrial 1-C-Grx1 was shown to coordinate [2Fe–2S] cluster *in vitro*, allowing it to participate in the ISC machinery. Aconitase, with a [4Fe–4S] cluster, is depicted as a representative holo-protein, which needs the involvement of Isa1, Isa2 and Iba57 for its maturation. B. ISC export machinery: It is comprised of the ABC transporter Atm of the mitochondrial inner membrane and the sulfhydryl oxidase Erv1 residing in the mitochondrial inter-membrane space. The trypanosome-specific dithiol trypanothione (TSH_2) most likely replaced glutathione (probable involvement: question mark). The unknown sulfur-containing substrate X–S is exported out by mitochondrial Atm. C. CIA machinery: A bridging [4Fe–4S] cluster is synthesized on the Cfd1/Nbp35 scaffold protein complex, with the dedicated aid of the early-acting electron transport chain Tah18/Dre2. Dre2, itself a Fe/S protein, coordinates one [4Fe–4S] and one [2Fe–2S] cluster. Subsequently, the bridging [4Fe–4S] cluster is transferred to the apo-proteins *via* intermediate CIA component Nar1 (which contains two [4Fe–4S] clusters) and the late-acting CIA targeting complex composed of Cia1, Cia2A, Cia2B and Mms19.

Out of four Fe/S protein biosynthesis systems classified in eukaryotes, *T. brucei* harbors mitochondrial iron–sulfur cluster assembly (ISC), ISC export machinery and cytosolic iron–sulfur protein assembly machinery (CIA). ISC machinery is responsible for all mitochondrial, cytosolic and nuclear Fe/S protein maturation, while the ISC export and CIA machinery are required for the cytosolic and nuclear Fe/S protein maturation [41].

Despite general conservation of these pathways, some interesting departures from the typical eukaryotic pathways were identified in the evolutionary divergent *T. brucei* [9,41–43].

3.1.1. ISC machinery

The *T. brucei* ISC system (Fig. 2A) is initiated by the cysteine desulfurase *Tb*Nfs–*Tb*Isd11 and the scaffold *Tb*IscU units. Both have been shown to be essential for the activity of mitochondrial and cytosolic aconitases, proving their role in Fe/S protein maturation [44,45]. RNAi-mediated down-regulation of *Tb*Nfs and *Tb*IscU in PS trypanosomes resulted in a dramatic increase in the amount of pyruvate as the metabolic end-product and a substantial drop in the ATP production. Following these changes, the PS mitochondrion in fact mimics its counterpart in the BS [44]. Recently, a dual localization of *Tb*Nfs has been reported in mitochondrion and nucleolus [46], though its exact function in the latter compartment remains to be established. Moreover, *Tb*Nfs and *Tb*Isd11 are also involved in tRNA thiolation [45] which connects Fe/S protein biogenesis and tRNA-modification adding sulfur [47]. *T. brucei* also encodes a Nfs-like selenocysteine lyase (*Tb*SCL), which is localized in the cytosol and nucleus [48]. Available data indicate that *Tb*Nfs can fully complement the function of *Tb*SCL but not *vice versa* [48].

Another key component of ISC is frataxin (*Tb*Fxn) (Fig. 2A), which is essential in the PS [49]. Several rescue studies performed in *T. brucei* with frataxin homologs from evolutionary unrelated organisms, proved its remarkable conservation. Overexpressed frataxin of the human parasite *T. vaginalis* carrying its specific hydrogenosomal signal was shown to be imported into the mitochondrion of PS and almost completely rescued the *Tb*Fxn RNAi phenotype [49]. Furthermore, frataxin homologs of the plant *Arabidopsis thaliana* and the marine diatom *Thalassiosira pseudonana* equipped with their own mitochondrial signals were efficiently imported into the PS mitochondrion [50]. Finally, human frataxin was processed in trypanosomes in a similar fashion as in human cells [51]. The electron-transfer chain, necessary for the maturation of Fe/S proteins, is composed of ferredoxin (*Tb*Fdx) and ferredoxin reductase (*Tb*FdR) (Fig. 2A). Two copies of *Tb*Fdx are present in the in *T. brucei* genome (for their functions see Section 3.2). The functional study of *Tb*FdR is yet to be performed.

The *T. brucei* ISC system also contains the essential chaperone system composed of *Tb*Hsp70, *Tb*Mge1 and *Tb*Jac1 (Fig. 2A) [9, 52, & Týč et al. unpubl. results], as well as the *Tb*Isa1 + 2 proteins that are required for the transfer of Fe/S clusters to a subset of apo-proteins [53]. Two more proteins were shown to be involved in the trypanosomal mitochondrial Fe/S protein biosynthesis. First, the monothiol glutaredoxin *Tb*1-C-Grx1 which binds an Fe/S cluster *in vitro* and was demonstrated to be involved in the parasite infectivity (Fig. 2A) [54] and second, the unique kinetoplastid-specific trypanothione (TSH_2) (Fig. 2B) [55].

3.1.2. ISC export machinery

The entire ISC export machinery is present in *T. brucei* (Fig. 2B), the main player being the mitochondrial inner membrane-associated ABC transporter *Tb*Atm (Fig. 2B). RNAi ablation of *Tb*Atm leads to the down-regulation of cytosolic CIA machinery with an unsettled Fe/S-cluster-incorporation into the cytosolic proteins. Enzymatic activities of marker Fe/S-proteins aconitase and fumarase decreased in the cytosol by 60% and 50%, respectively, but the same activities in the mitochondrion stayed unchanged. Furthermore, depletion of *Tb*Atm disrupted the cytosolic, but not mitochondrial tRNA thiolation [56]. In yeast and in *A. thaliana*, Atm was implicated in the transport of glutathione polysulfide from the mitochondrion [57], and it is plausible that different forms of thiols may represent the substrate for Atm also in *T. brucei* (see Section 3.2).

Another component is *Tb*Erv1, a protein crucial for both life stages (Fig. 2B) [42]. Erv1 is involved in another essential process, the mitochondrial intermembrane space import and assembly (MIA) pathway [58,59]. To accomplish this pathway, Erv1 collaborates with Mia40 for import and oxidative folding of cysteine-rich proteins into the mitochondrial inter-membrane space. This fundamental eukaryotic process is divergent in trypanosomes and their close evolutionary relatives, as Erv1 functions in the absence of the Mia40 protein [60,42]. Biochemical analysis of recombinant and mutagenized trypanosome Erv1 demonstrated that *Tb*Erv1 is capable of passing electrons to both oxygen and cytochrome *c*, which is compatible with the physiology of the parasite in its mammalian host and insect vector [42].

3.1.3. CIA machinery

The CIA machinery matures the cytosolic and nuclear Fe/S proteins along with the ISC and ISC-export systems, which among other proteins, include essential components of the translation system, DNA synthesis and repair. Detailed organization of the CIA machinery in model eukaryotes has been described in recent reviews [40,41,61].

Depletion of the *T. brucei* CIA components individually by RNAi revealed the dispensability of most of them, with the exception of the scaffold proteins *Tb*Nbp35 and *Tb*Cfd1 (Fig. 2C) [43,62]. However, a simultaneous depletion of two components of the early or intermediate part of the CIA pathway, invariably affected the viability of both life stages. It indicates that when just a single component of a module (here, the modules are 'electron transport chain', 'scaffold', and the 'targeting complex') is depleted, its interacting partner seems to partially complement the absence [43].

Biochemical characterization revealed that *Tb*Tah18, a component of the early-acting 'electron transport chain module,' is like its yeast, human and plant homologs a diflavin reductase. However, unlike its human and yeast counterparts, the other component of this module, *Tb*Dre2, lacks the entire N-terminal *S*-adenosylmethionine methyltransferase-like domain, while the Fe/S cluster-binding domain is retained. This truncated structure is a common character of all kinetoplastid flagellates [43].

The degree of functional conservation of the *T. brucei* CIA factors has been put to test by the yeast complementation assays. When (over) expressed individually, neither *Tb*Dre2 nor *Tb*Tah18 could rescue the absence of respective yeast proteins. Since in the plant and human cells, Dre2 and Tah18 tightly interact [63,64], the complementation assays were performed in the background of a double yeast mutant, which was rescued by co-expressed *T. brucei* orthologues. The complementation also partially rescued activity of isopropylmalate isomerase, a cytosolic Fe/S cluster-containing protein [43].

T. brucei also possesses two Cia2 proteins (*Tb*Cia2A and *Tb*Cia2B) forming the late-acting 'targeting-complex' module along with *Tb*Cia1 & *Tb*Mms19 (Fig. 2C) [43, & S.B. and J.L., unpubl. results]. The differential target specificity of human CIA2A and CIA2B [65] encourages future studies on this module in the evolutionary distant *T. brucei*. While CIA2A is reported to mature the human IRP1, a protein involved in cellular iron-homeostasis [65], it would be of interest to investigate the targets of *Tb*Cia2A in the IRP1-lacking trypanosome.

3.2. Heme

Heme, an iron-containing porphyrin, is a prosthetic group involved in numerous key cellular processes in an absolute majority of extant life [66–68]. Due to its capacity to transfer electrons and bind diatomic gases, heme has been implicated in oxidative metabolism such as electron transport-dependent oxidative phosphorylation, oxidative stress

response, oxygen transport, sensing and detoxification. It is also used as a signaling molecule in many aerobic organisms [66,67].

There are three biologically important forms of heme (types *a*, *b*, and *c*) which differ by modifications in the porphyrin ring. The most common type is heme *b* (or protoheme), from which the two other forms of heme are formed [69]. Most eukaryotes are able to synthesize heme *b* from δ-aminolevulinic acid through seven universally conserved enzymatic steps [70], although several exceptions are known [71,72]. Trypanosomes and related kinetoplastid flagellates feature prominently among them, as they belong to a small group of eukaryotes that are unable to synthesize heme *de novo* and hence, totally rely on external heme [68]. As heme auxotrophs, these parasites acquire this prosthetic group from the invertebrate vector or the final vertebrate host. Interestingly, there is a fundamental difference in the way trypanosomes acquire heme in different life cycle stages. It was shown previously that the haptoglobin–hemoglobin (HpHb) complex is the main source of heme for the BS cells [73]. This complex is internalized *via* endocytosis by the haptoglobin–hemoglobin receptor (HpHbR), which is exclusively expressed in BS and missing from the PS flagellates (Fig. 1A). Moreover, it was also demonstrated that HpHbR does not have the capacity to internalize free heme. The PS cells are able to acquire free heme, but not *via* the HpHb complex [73] (Fig. 1B). Instead they employ a membrane transporter called *Tb*Hrg (Fig. 1B), the homolog of which was so far functionally studied in the related human pathogen *Leishmania* [74,75]. It seems that in *T. brucei* it functions as the free-heme transporter inversely to HpHbR, as it is expressed exclusively in the PS stage (E.H. and J.L., unpubl. results).

It is worth mentioning that the BS cells in which HpHbR was knocked out are still able to proliferate *in vivo* and *in vitro* [73], possibly suggesting the rather counterintuitive dispensability of heme by this blood-dwelling parasite. The plausibility of such a scenario is strengthened by the fact that the related plant pathogenic flagellate *Phytomonas serpens* is able to incorporate heme when available, yet lacks most hemoproteins, and can survive in total absence of this otherwise essential cofactor [76].

Both the mammalian- and insect-parasitizing BS and PS of *T. brucei*, respectively, occupy an environment rich in hemoglobin, which is permanently available in human blood and temporarily present in the arthropod vector after it has ingested large quantities of blood. Therefore, heme uptake and utilization by trypanosomes have to be tightly regulated [77]. In any case, while we are beginning to understand the import of heme and its metabolism in the cytosol of trypanosomes, its import into the mitochondrion and other compartments remains enigmatic.

Upon acquisition by the trypanosome, heme is incorporated into hemoproteins, mainly the cytochromes that are core components of the respiratory chain, which is known to contain all types of heme groups. The best-known components are the inter-membrane space-situated *c*-type cytochromes cyt *c* and cyt *c*1 with a kinetoplastid-specific form of heme attachment to a single cysteine residue at the heme-binding motif [78,79]. On the genomic level, trypanosomes lack a recognizable cyt *c* biogenesis system, which implies that a highly divergent machinery is responsible for this unique attachment of heme, which in all other eukaryotes occurs *via* one of only two known distinct pathways [79,80].

The unique mode of heme attachment in cytochromes (cyt *c* and cyt *c*1), and the absence of any known biogenetic genes, suggested the presence of a novel *c*-type cytochrome biogenesis machinery (system V) in the mitochondrion of trypanosomes. It is highly divergent from the known systems, since no components have been identified yet [78].

Heme *a* is required for the activity of respiratory complex IV (cytochrome *c* oxidase), while complex II (succinate dehydrogenase) incorporates heme if available, yet was shown in the related *P. serpens* to be fully functional even in its absence [76].

Heme *a* is synthesized from heme *b* by two enzymatic steps involving heme *o* synthase (cox10) and heme *a* synthase (cox15). Both genes are conserved in trypanosomatids and have been studied in detail in *Trypanosoma cruzi* [81]. It was suggested that ferredoxin (Fdx) and ferredoxin reductase (FdR) function simultaneously with cox15 in the hydroxylation of heme *o* to form heme *a* [82]. Out of two *T. brucei* Fdxs, only *Tb*FdxA appears to be essential for this process, while the other homolog known as *Tb*FdxB is dispensable and its function remains unknown [83]. Moreover, both human mitochondrial ferredoxins successfully rescued the PS flagellates depleted for *Tb*FdxA, testifying to the high functional conservation of these ubiquitous proteins throughout eukaryotes [83]. By participating in heme *a* synthesis as well as in Fe/S biogenesis, ferredoxins bridge these two iron-dependent core cellular processes. Another protein involved in both processes seems to be ABCB transporter *Tb*Mdl, which works in concert with *Tb*Atm in order to establish the homeostasis of iron cofactors in the organelle. It was shown recently that *Tb*Mdl plays a role in heme import into the mitochondrion of *T. brucei*, since organellar heme *b* and *a* dropped to great extent in flagellates depleted for *Tb*Mdl. On the other hand, the amount of heme *a* and *b* in *Tb*Atm RNAi knock-down was not significantly changed, suggesting that it may be involved in heme homeostasis indirectly, possibly by increasing thiols in the cytosol, which may lead to heme degradation [56].

3.3. Non-heme and non-Fe/S iron

Apart from the discussed essential iron cofactors, a third type, the so-called non-heme-non-Fe/S iron, is involved in catalytic activities within the mitochondrion. The non-heme-non-Fe/S iron pool can be measured by a dedicated assay [84]. Several proteins belonging to the above-mentioned category are essential components of the *T. brucei* mitochondrion, namely superoxide dismutase (SOD), ribonucleotide reductase (RNR) and the trypanosome alternative oxidase (TAO) [85–87]. The SODs assist aerobic organisms to withstand oxygen toxicity [88]. *T. brucei* possess four SODs; *Tb*SODA and *Tb*SODC are mitochondrial proteins, while *Tb*SODB1 resides in the cytosol and *Tb*SODB2 is predominantly glycosomal [85,89]. Ribonucleotide reductase actuates the formation of deoxyribonucleotides from ribonucleotides, a crucial step towards DNA synthesis [90]. *T. brucei* retains the conserved eukaryotic class-I RNR containing subunits R1 and R2 [86]. The *Tb*RNR is developmentally coordinated by post-transcriptional regulation of the R2 subunit [91].

For their energy production, the BS trypanosomes depend exclusively on glycolysis, which they have largely compartmentalized into the dedicated organelles called the glycosomes [11,92]. In an alternative BS-specific respiratory chain, glycerol-3-phosphate dehydrogenase supplies electrons to TAO, which subsequently delivers them to oxygen, and re-oxidizes NADH produced by glycolysis [87]. Since the mammalian hosts do not possess this protein, it is highly contemplated as a potential drug target [93,94]. Ascofuranone, a compound isolated from the phytopathogenic fungus *Ascochyta visiae*, proved to be an effective inhibitor of TAO [93]. A recent structural analysis of TAO revealed the character of the ascofuranone-binding site [94].

TAO belongs to the non-heme di-iron carboxylate protein superfamily, composed of several groups of mono-oxygenases, oxidases, and dioxygen transport and sensing proteins [95]. It has a specific di-iron-carboxylate active site that contains two non-heme irons linked by at least one carboxylate-bridging amino acid residue [96]. *In vitro* studies with iron-chelating agents combined with mutagenesis of the TAO protein established its functional dependency on iron [96,97]. The recently solved crystal structure of TAO represents first such case among alternative oxidases [94]. It revealed that the asymmetric TAO unit is formed by two monomers each of which is composed of a long N-terminal arm and six long and four short α-helices, which participate in holding the di-iron active center located deep inside the TAO molecule [94]. It is hoped that structural determination of TAO will accelerate the possibilities to exploit its function for therapeutic interventions.

4. Iron: pathogenesis and cure

Transition metals are essential elements of life, with iron standing out as an element involved in innumerable biological events. Iron is one of the quintessential factors involved in host-pathogen interaction, where a constant duel in sequestering this vital metal is taking place [4, 98]. Indeed, iron plays one of the central roles in the pathogenesis of trypanosomes, as their mammalian host tries to curb its availability [99,100].

A key trait of trypanosome pathogenesis is a hyper-activation of macrophages, followed by increased phagocytosis of erythrocytes eventually leading to anemia. This phenomenon creates low iron conditions, a true challenge for the blood-dwelling parasites to sustain the infection [99,101]. At the same time, the mammalian immune system also plays a role in regulating iron availability by specific mechanisms involving cytokines and hormones [100]. The hepatocyte-secreted peptide hormone hepcidin helps to create iron-depleted conditions [102]. It down-regulates the iron exporter ferroportin, thus affecting the cellular iron export to the bloodstream [103]. Based on these data, chelation of iron seems to be an effective strategy for the development of new trypanosomal drugs. Compounds showing strong affinity to iron can be isolated from bacteria and fungi [104]. Deferoxamine secreted by *Streptomyces pilosus* is an example of such an iron chelator [105]. Deferoxamine chelates cellular iron, hence preventing its incorporation into newly translated apoproteins [106]. A deferoxamine-based drug named Desferal® is used for the treatment of acute iron toxicity and chronic iron overload (Novartis Pharmaceuticals Corporation, 2007). Moreover, deferoxamine has a trypanocidal activity against BS *T. brucei*, since the flagellates are 10-fold more sensitive to it than the mammalian cells [106]. Likewise, compounds that contain the hydroxamic acid moiety form strong complexes with a variety of metals, specifically iron. For example salicyl hydroxamic acid (SHAM), a hydroxamate analog, represents a competent inhibitor of *T. brucei* [107].

5. Conclusions and future avenues

In this review we covered the iron-associated biology of *T. brucei*. The involvement of iron in several crucial biological functions makes it an essential micronutrient. Substantial reliance of trypanosomes on iron sources appears to be a possible Achilles heel of these serious human pathogens.

From an evolutionary point of view, these parasites developed unique methods to sequester iron. Proteins involved in these processes are consequently regarded as cornerstones of a typical host-parasite interaction. Henceforward, they are ideal targets for future drugs. The trypanosome-specific transferrin receptor (TfR) is a particularly promising one. Moreover, special attention should be given to the iron chelators, although their potential as antiparasitic drugs has yet to be weighed.

Several areas of trypanosomal Fe/S protein biosynthesis are yet to be addressed, especially the CIA targeting complex and its possible substrates. In any case, these ancestral protists seem to retain a highly conserved form of this essential biosynthetic process, which limits potential for drug development. The same applies for the heme acquisition pathways, which in this heme auxotroph, do not seem to qualify for a promising drug target [68].

Acknowledgments

This research was supported by the Czech Grant Agency P305/14/23986S and P305/12/2261, grant of the Czech Academy of Sciences M200961204, the Bioglobe grant CZ.1.07/2.3.00/30.0032, the COST Action CM1307, and the Praemium Academiae award to J. L. We acknowledge the use of research infrastructure that has received funding from the EU 7th Framework Programme under grant agreement No. 316304.

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