Knock-downs of Iron-Sulfur Cluster Assembly Proteins IscS and IscU Down-regulate the Active Mitochondrion of Procyclic Trypanosoma brucei

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Transformation of the metabolically down-regulated mitochondrion of the mammalian bloodstream stage of Trypanosoma brucei to the ATP-producing mitochondrion of the insect procyclic stage is accompanied by the de novo synthesis of citric acid cycle enzymes and components of the respiratory chain. Because these metabolic pathways contain multiple iron-sulfur (FeS) proteins, their synthesis, including the formation of FeS clusters, is required. However, nothing is known about FeS cluster biogenesis in trypanosomes, organisms that are evolutionarily distant from yeast and humans. Here we demonstrate that two mitochondrial proteins, the cysteine desulfurase TbiscS and the metallochaperone TbiscU, are functionally conserved in trypanosomes and essential for this parasite. Knock-downs of TbiscS and TbiscU in the procyclic stage by means of RNA interference resulted in reduced activity of the marker FeS enzyme aconitase in both the mitochondrion and cytosol because of the lack of FeS clusters. Moreover, down-regulation of TbiscS and TbiscU affected the metabolism of procyclic T. brucei so that their mitochondria resembled the organelle of the bloodstream stage; mitochondrial ATP production was impaired, the activity of the respiratory chain protein complex ubiquinol-cytochrome-c reductase was reduced, and the production of pyruvate as an end product of glucose metabolism was enhanced. These results indicate that mitochondrial FeS cluster assembly is indispensable for completion of the T. brucei life cycle.

Trypanosoma brucei is one of the most important protozoan pathogens, responsible for human sleeping sickness and nagana in livestock. Moreover, because the genome of T. brucei has recently been completely sequenced (1), and the cells are amenable to approaches of involving reverse genetics (2), T. brucei has become a new model organism, which is evolutionarily highly divergent from classical models such as Saccharomyces cerevisiae. While yeast and other fungi are more related to metazoa including humans (eukaryotic group Opisthokonta), trypanosomatids belong to the distant eukaryotic group called Excavata (3, 4). This group is formed exclusively of unicellular eukaryotes, many of them with highly modified mitochondria (5). The mitochondrion of T. brucei is of particular interest, because it undergoes dramatic metabolic and structural changes during the cell cycle between the blood of the mammalian host and the digestive tract of the tsetse fly. An excess of glucose in the mammalian host permits the bloodstream stage to employ glycolysis for energy generation, a significant part of which is localized to specialized peroxisomes called glycosomes (6), producing pyruvate as a major end product. Consequently, the mitochondrion lacks the cytochrome-dependent electron transport chain and the activities of the citric acid cycle enzymes and is thus impaired in its ability to produce ATP by oxidative phosphorylation (7). Through its functions as a sink for reducing equivalents from glycolysis, the mitochondrion still plays an indispensable role in the metabolism of blood stages (8). In the insect vector, trypanosomes encounter a nutrient-poor environment, where they can only survive through an overall switch in their energy metabolism, primarily by activating the mitochondrion. In the active mitochondria of procyclics, pyruvate is degraded with the benefit of additional ATP synthesis, mainly to acetate and succinate (9). However, most of the excreted succinate is produced in glycosomes (10). This transformation is accompanied by the de novo synthesis of iron-sulfur (FeS) clusters and maturation of a number of FeS proteins including electron-transporting subunits of the mitochondrial respiratory chain (complexes I, II, and III) and aconitase, an enzyme with dual localization in the mitochondrion and cytosol (9). The biogenesis of FeS clusters is a recently discovered process essential for both prokaryotic and eukaryotic cells (11, 12). In eukaryotes, the de novo formation of FeS clusters was first discovered in mitochondria (13) and later in other organelles of the cell.

The abbreviations used are: FeS, iron-sulfur; RNAi, RNA interference; HPLC, high pressure liquid chromatography; MRP2, mitochondrial RNA-binding protein 2.

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endosymbiotic origin including hydrogenosomes, mitosomes, and plastids (14–18). The machinery responsible for mitochondrial FeS cluster assembly consists of at least 10 different proteins, the key components being pyridoxal 5-phosphate-dependent cysteine desulphurase IscS, which generates sulfur (S0) from cysteine, and the metallochaperone IscU, which provides the molecular scaffold for the formation of a transient FeS cluster (11). The transient FeS cluster is transferred from IscU to apoproteins during FeS protein maturation (19).

In S. cerevisiae, mitochondria were shown to play an essential role not only in the biosynthesis of mitochondrial FeS proteins but also in the maturation of FeS proteins localized in the cytosol and nucleus. For example, this essential requirement for the FeS cluster assembly machinery was demonstrated for Ril1, a cytosolic FeS protein that is indispensable to ribosomal functionality (19, 20). A different model was proposed for the biogenesis of FeS clusters in human cells. In addition to mitochondria, several components of the mitochondrial FeS cluster assembly machinery were detected in the cytosol and nucleus (21–23), and the ability of their cytosolic forms to promote de novo FeS cluster formation was demonstrated (24).

Because no information is available on FeS cluster assembly in trypanosomes, we investigated the function of mitochondria of T. brucei procyclins in this process. Initially, we identified the main components of its FeS cluster assembly machinery and constructed cell lines in which the expression of the IscS and IscU genes was down-regulated by means of RNA interference (RNAi). Analyses of the resulting phenotypes provide the first insight into FeS cluster biogenesis in the mitochondria of parasitic protists and support the hypothesis that the mitochondrial plays a fundamental and evolutionary conserved role in cellular FeS cluster assembly throughout the eukaryotes.

EXPERIMENTAL PROCEDURES

Construction of Vectors, Transfection, Cloning, RNAi Induction, and Growth—A 424-bp fragment of the TbiscS2 gene (supplemental material) was amplified by PCR from the T. brucei 427 genomic DNA using oligonucleotides IscS-F1 (5′-CACCATATGGTAGAGATGAAGCGTGATT) and IscS-R1 (5′-CACAAGCTTTCCTTCATCACGAAGT) (added NdeI and HindIII restriction sites are underlined), was cloned into pCR2.1 TOPO (Invitrogen) and subcloned in pZJM (25). Three sequenced clones were identical.

Reverse Transcription-PCR Analysis and Northern Blotting—DNA enriched for TbiscS2 mRNA was synthesized by reverse transcription of poly(A)+ RNA with IscS-R2 (5′-CGAATTA-AATCTGCACTTTGCGTGC) and used as a template for amplification of the 5′ end region of this mRNA with IscS-R3 (5′-GGCAATATTGGTGGGACTCCGGTTG) and the upstream spliced leader-specific primer SLTb1 (5′-AUCTAACCCTAT-TATTAGAACAGT). Three sequenced clones were identical.

Total RNA was isolated from 5 × 107 exponentially growing noninduced and RNAi-induced cells by extraction with Tri Reagent (Sigma-Aldrich). TbiscS2 or TbiscU gene probes were labeled by random priming with [α-32P]dATP (MP Biomedicals, Irvine, CA). Hybridization was carried out using standard procedure (28). The radioactive signal was detected using storage phosphorimaging.

Preparation of Antibodies and Immunoblot Analysis—The coding region of TbiscS2 and TbiscU was subcloned into a pQE30 vector (Qiagen) incorporating an N-terminal His6 tag. Soluble protein was obtained from induced bacterial cells under denaturing conditions using affinity chromatography as described in the manufacturer’s instructions. Polyclonal antibodies against recombinant TbiscS were prepared by immunizing rabbits (28, 29). The polyclonal rabbit antibodies raised against recombinant TbiscU were commercially prepared (Seva-Imuno, Prague, Czech Republic). Rabbit γ-globulins were purified from sera by affinity chromatography in a Prosep®, a high capacity column (Bioprocessing Ltd., Consett, UK).

Total cell lysates and subcellular fractions of noninduced and induced trypanosomes were separated on SDS-PAGE gels, blotted, and probed with polyclonal antibodies against TbiscS2, TbiscU, aconitase (Ref. 40; provided by M. Boshart), Hsp60 (provided by P. A. M. Michels), mitochondrial RNA-binding protein 2 (MRP2) (28), and the Lp protein (29). Secondary anti-rabbit or anti-mouse antibodies coupled to alkaline phosphatase (MP Biomedicals) were visualized with 5-bromo-4-chloro-3-indolyl phosphate (Sigma-Aldrich).

Digitonin Fractionation—Digitonin fractionation of procyclins was performed as described elsewhere (30) with the following modifications. The cells were washed twice and resuspended in ice-cold SHE buffer (25 mM Hepes, pH 7.4, 250 mM sucrose, 1 mM EDTA) at ~5 × 107 cells/ml. Aliquots containing 1 mg of protein were resuspended in 350 μl of Hanks’ balanced salt solution buffer (Invitrogen) and incubated for 4 min at 25 °C with increasing concentrations of digitonin (Merck) dissolved in dimethylformamide and centrifuged at 14,000 × g for 2 min. Whole cell lysates were prepared by incubating the same aliquots in 350 μl of Hanks’ balanced salt solution buffer containing 0.1% Triton X-100 for 5 min on ice and centrifuged as above. The resulting supernatants were immediately assayed for the presence of the cytosolic (pyruvate kinase) and mitochondrial (threonine dehydrogenase) marker enzymes. To obtain cytosolic and mitochondria-rich fractions, the concentration of digitonin was used that released the maximum pyruvate kinase activity and no threonine dehydrogenase into the supernatant. This supernatant was considered to represent the
cytosolic fraction. Pelleted intact mitochondria were washed once and then resuspended in 350 μl of Hanks’ balanced salt solution buffer and incubated with 0.1% Triton X-100 for 5 min on ice. After centrifugation, the supernatant containing mitochondrial matrix proteins was collected (the mitochondrial fraction).

**Enzyme Assays and Determination of Metabolic End Products**—The activities of pyruvate kinase and threonine dehydrogenase were monitored spectrophotometrically at 340 nm as a rate of NADH oxidation or NAD reduction, respectively (31, 32). The activity of aconitase was measured as the production of cis-aconitate, monitored at 240 nm (33). Ubiquinol-cytochrome-c reductase activity (complex III) was measured in QCR buffer (40 mM sodium phosphate buffer, pH 7.4, 0.5 mM EDTA, 20 mM sodium malonate, 50 μM horse heart cytochrome c (Sigma-Aldrich), 0.005% dodecylmaltoside) as the rate of 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinol oxidation monitored at 550 nm (prior to use, decylubiquinone (Sigma-Aldrich) was reduced as described elsewhere (34)). KCN was added to a final concentration of 200 μM as an inhibitor of interfering oxidase activity.

To determine the metabolic end products, −8 × 10^7 cells were washed once with phosphate-buffered saline, resuspended in 200 μl of incubation buffer (phosphate-buffered saline buffer supplemented with 11 mM glucose and 24 mM NaHCO₃, pH 7.3), and incubated for 2 h at 27 °C. After centrifugation for 10 min at 1,400 × g, the supernatant was analyzed by HPLC in a PL Hi-Plex H column as described elsewhere (35).

**ATP Production in Isolated Mitochondria**—Intact mitochondria were prepared from cell aliquots containing 1 mg of protein by digitonin fractionation as described above. The mitochondrial pellet was resuspended in 750 μl of the buffer for *in organello* ATP production assay (20 mM Tris-HCl, pH 7.4, 15 mM KH₂PO₄, 0.6 mM sorbitol, 10 mM MgSO₄, 2.5 mg/ml bovine serum albumin), and 75-μl aliquots were used for each measurement. ATP production was induced by the addition of substrates as published elsewhere (36). The concentration of ATP was determined by a luminometer using the ATP Bioluminescence assay kit CLS II (Roche Applied Science).

**Measurement of Mitochondrial Membrane Potential**—Tetramethyrlodamine ethyl ester (Molecular Probes, Eugene, OR) uptake was used as a measure of the mitochondrial membrane potential (37).

**EPR Analysis of FeS Clusters**—EPR spectra were recorded on a Bruker Elexys E580 spectrometer operating in X-band continuous-wave mode, with an Oxford Instruments ESR9000 liquid helium flow cryostat. The measurement conditions were: temperature, 12 K; microwave power, 20 milliwatts; frequency, 9.38 GHz; and modulation amplitude, 1 millitesla.

**RESULTS**

**Genes Coding for TbiscS and TbiscU**—The *T. brucei* genome project data base (www.sanger.ac.uk/) was searched for homologs of IscS and IscU using *S. cerevisiae* Nfs1p and Isu1p as queries. A BLAST search for IscS identified two homologs in the *T. brucei* genome, here named TbiscS1 and TbiscS2, with calculated molecular weights of 48,991 and 48,150, respectively. Alignment of their deduced amino acid sequences together with Nfs1p revealed significant differences between the two trypanosome genes (supplemental material). Similar to Nfs1p, TbiscS2 possesses conserved residues corresponding to the active site loop that are responsible for the targeted delivery of sulfur to IscU (38), as well as the conserved C terminus essential for specific interaction with IscU (39). In contrast, TbiscS1 lacks these regions. Phylogenetic analysis of the two *T. brucei* proteins indicated that TbiscS2 is closely related to other mitochondrial IscS homologs, whereas TbiscS1 clusters together with putative selenocysteine lyases (data not shown). Thus, TbiscS2 was selected for further studies. To prove that TbiscS2 is transcribed, the spliced leader RNA and primers from the 5′ end of the gene were used for reverse transcription-PCR. In three clones with identical sequences, the splice acceptor site was mapped to 25 bp upstream of the initial methionine, resulting in a very short 5′-untranslated region (data not shown).

A BLAST search for Isu1p identified a single IscU homolog (TbiscU) in the *T. brucei* genome data base with a calculated molecular weight of 19,435. Alignment of the deduced TbiscU amino acid sequence with yeast Isu1p revealed that all three conserved cysteine residues required for the assembly of the transient FeS cluster are present in TbiscU (supplemental material).

**TbiscS2 and TbiscU Are Localized in the Mitochondrion**—PsortII analysis (psort.nibb.ac.jp/) of TbiscS2 and TbiscU predicted N-terminal leader sequences for targeting the proteins into mitochondria as well as putative cleavage sites with the characteristic arginine at the −2 position that is recognized by mitochondrial processing peptidase (supplemental material). To verify the predicted cellular localization of these proteins in procyclic *T. brucei*, specific polyclonal antibodies were raised against recombinant TbiscS2 and TbiscU and used for immunoblot analysis. In subcellular fractions obtained by differential permeabilization of the cell membranes by increasing concentrations of digitonin (30), both TbiscS2 and TbiscU were detected in the mitochondrial fractions, whereas no signals were observed in the cytosol (Fig. 1). Antibodies against the MRP2 (28) and the cytosolic La protein (29) were used as controls in cell fractionation experiments. The low cellular abundance of TbiscS2 is probably responsible for difficulty in identifying the protein in the whole cell lysate (40).

**Inhibition of TbiscS2 and TbiscU Gene Expression by RNAi**—A 424-bp-long fragment of TbiscS2 and a 484-bp-long fragment of TbiscU (supplemental material) were cloned into the

![FIGURE 1. Subcellular localization of TbiscS2 and TbiscU by immunoblot analysis in whole cell lysates, cytosol, and mitochondrion of the parental 29-13 cells. MRP2 and La proteins were used as mitochondrial and cytosolic marker proteins, respectively.](image-url)
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FIGURE 2. Effect of TbiscS2 (A) and TbiscU (B) RNAi on mRNA levels. TbiscS2 and TbiscU mRNA levels were analyzed by Northern blot analysis in extracts from parental strain 29-13 (WT), noninduced cells (day 0) and in extracts isolated 2, 4, and 6 days after RNAi induction (A, TbiscS RNAi cells) or 2 and 4 days after RNAi induction (B, TbiscU RNAi cells). The positions of the targeted mRNAs and the double-stranded RNAs synthesized after induction are indicated with arrows. WT, wild type.

FIGURE 3. Effect of TbiscS2 (A) and TbiscU (B) RNAi on cell growth. The cell density of the parental 29-13 cells (triangles), noninduced cells (diamonds), and cells induced with 2 μg/ml of tetracycline (squares) are indicated. The y axis is a log scale and represents the product of the measured cell densities and total dilution. The arrows indicate the time points chosen for biochemical experiments. The insets show a Northern blot of the total RNA (top panels) and immunobLOTS of mitochondrial fractions (middle and bottom panels) before (−) and after (+) RNAi induction by tetracycline. The mitochondrial protein MRP2 was used as a protein loading control.

FIGURE 4. Decrease in activity of marker FeS enzyme aconitase in TbiscS2 (A) and TbiscU (B) RNAi cells. The percentages of specific activity of aconitase in total cell lysate, cytosol, and mitochondria in cells before (−tet) and after (+tet) RNAi induction by tetracycline are shown by black and gray columns, respectively. Immunoblot analyses of aconitase apoprotein in the respective fractions are shown below the bars. The mitochondrial non-FeS protein Hsp60 was used as a protein loading control. The bars indicate standard deviations (n = 3).

TbiscU are required for FeS cluster assembly in the trypanosome organelle and whether the mitochondrial FeS cluster assembly machinery is also involved in the maturation of cytosolic FeS proteins. In procyclic trypanosomes, aconitase has dual localization, with 70 and 30% of its total active form being distributed in the mitochondrion and cytosol, respectively (33). After RNAi induction, the activity of aconitase was reduced in both cellular compartments (Fig. 4). The TbiscS2 knock-down cells displayed higher inhibition of the cytosolic aconitase activity, which was down to ~30% of that in the noninduced cells, than mitochondrial aconitase, which retained ~70% of its activity (Fig. 4A). The down-regulation of TbiscU resulted in a considerably higher inhibition of aconitase activity in the mitochondrion than in the cytosol (Fig. 4B). In contrast, the activity of pyruvate kinase, a cytosolic enzyme that does not require an FeS cluster for its activity, remained unaffected (data not shown).

In addition to the impaired FeS cluster assembly, this inhibition of aconitase activity could be caused by affecting the expression of the corresponding protein or by oxidation of the [4Fe4S] cluster of the active aconitase to its inactive [3Fe4S] form (41). To exclude these possibilities, the protein level and presence of aconitase clusters in the subcellular fractions of TbiscS2-induced cells were verified by immunoblot analysis (Fig. 4) and EPR (Fig. 5). No decrease in the aconitase level in the cytosol of the induced cells was observed on immunoblots. The EPR analysis unequivocally confirmed that the decrease in the cytosolic aconitase activity was a consequence of decreased FeS cluster formation. Because the [4Fe4S] cluster is highly susceptible to conversion to [3Fe4S] upon exposure to oxygen, we assumed that in the samples prepared under aerobic conditions, all of the aconitase clusters were present in the [3Fe4S] form. The signal corresponding to total [3Fe4S] clusters of cytosolic aconitase in the induced cells was only 20% of that in the noninduced cells (Fig. 5A).

The level of mitochondrial aconitase detected on immunoblots of TbiscS2 knock-downs was slightly decreased in comparison to noninterfered cells. This effect is most likely caused by the degradation of apo-aconitase, which was observed in yeast mitochondria (42). The mitochondrial EPR spectra comprise contributions from the [3Fe4S] clusters of aconitase (peak at
As expected, the intensity of the combined signals in the mitochondria of induced cells was decreased, accounting for about 30% of that in noninduced cells (Fig. 5B). These results indicate that TbiscS2 and TbiscU, both mitochondrial components of the FeS cluster assembly machinery, are essential for the maturation of FeS proteins operating in the mitochondrion as well as in the cytosol of T. brucei procyclins.

Metabolic Changes Mimic Interstargial Transformation—FeS proteins are involved in critical steps of mitochondrial energy metabolism, which is substantially down-regulated in the bloodstream stages. Similar metabolic changes might be induced in cells with impaired FeS cluster formations, which in turn affect the function of FeS proteins. To test this hypothesis, we compared mitochondrial ATP synthesis, membrane potential, and the end products of glucose metabolism in FeS cluster assembly-competent cells and procyclins in which TbiscS2 and TbiscU were down-regulated.

The mitochondrial ATP of the procyclic stage is produced via three different pathways that can be assayed in the isolated intact mitochondria (36): (i) Succinate is the main substrate for oxidative phosphorylation. A respiratory chain loaded with electrons by succinate dehydrogenase generates a proton gradient that drives mitochondrial F0F1 ATP synthetase (45). This pathway involves at least two FeS proteins, namely succinate dehydrogenase and the Rieske protein of ubiquinol-cytochrome-c reductase. In TbiscS2 knock-downs, succinate dehydrogenase-dependent ATP production was completely lost (Fig. 6A). The residual succinate-dependent ATP synthesis was insensitive to malonate, a competitive inhibitor of succinate dehydrogenase. Accordingly, the specific activity of ubiquinol-cytochrome-c reductase in mitochondrial lysates of the parental strain and noninduced cells fluctuated around 750 milliunits/mg, whereas the level of FeS cluster assembly-impaired cells was reduced by about 50% in three independent experiments. (ii) α-Ketoglutarate induces ATP production by substrate level phosphorylation occurring in the citric acid cycle. (iii) Pyruvate and succinate induce ATP production by substrate level phosphorylation occurring in the acetate-succinate CoA transferase/succinyl-CoA synthetase cycle. Interestingly, both pyruvate-succinate- and α-ketoglutarate-induced ATP synthesis was moderately decreased upon down-regulation of TbiscS2, although no FeS proteins are directly involved in these pathways (Fig. 6, B and C). However, both pyruvate and α-ketoglutarate metabolism are indirectly dependent on the maintenance of the mitochondrial redox balance, for which FeS proteins are required.

Previous experiments indicated a malfunction of FeS protein-dependent components of the proton generating respiratory chain in FeS cluster assembly-impaired cells. Thus, changes in mitochondrial membrane potential can be expected. To study the mitochondrial membrane potential in T. brucei, tetramethylrhodamine ethyl ester has been evaluated as a sensitive fluorescence probe (37). Indeed, cytofluorometric profiles of trypanosomes using tetramethylrhodamine ethyl ester in this study indicated a decreased membrane potential in the iron-sulfur cluster assembly-impaired mitochondria of T. brucei interfered against TbiscS2 and TbiscU in comparison with the noninterfered cells (supplemental material).

All of these results indicate that the involvement of mitochondria in energetic metabolism is decreased
in TbiscS2 and TbiscU knock-downs. In the bloodstream stages, the inactivation of mitochondria is compensated by glycolysis producing pyruvate as a major metabolic end product. A similar trend was observed in FeS cluster assembly-impaired cells. The production of succinate decreased to 84% and 41% after induction of RNAi in TbiscS2 and TbiscU knock-down cells, respectively. The acetate production decreased to 54% after TbiscS2 and to 48% after TbiscU knock-down. In contrast, the production of pyruvate was 8-fold and more than 2-fold increased in TbiscS2 and TbiscU knock-down cells, respectively, when compared with wild-type trypanosomes (Fig. 7).

**DISCUSSION**

In this paper, we have demonstrated that TbiscS2 and TbiscU, homologs of the bacterial and yeast cysteine desulfurase IscS/Nfs, and the scaffold protein IscU/Isu are indispensable for FeS cluster formation and consequently the viability of the procyclic stage of *T. brucei*. Although both proteins were specifically localized to the mitochondrion, their deficient expression affected the maturation of FeS proteins operating not only in the mitochondrion, but also in the cytosol.

The mitochondria isolated from knock-down cells displayed decreased enzymatic activities of FeS proteins including aconitase and Rieske protein-containing ubiquinol-cytochrome-c reductase, undetectable succinate-induced ATP synthesis mediated by succinate dehydrogenase (complex II), and decreased mitochondrial membrane potential. In addition, the cells expressing the interfering RNA had decreased cytochrome-dependent respiration via complex IV and partially switched to the trypanosome alternative oxidase.\(^3\)

The dual localization of aconitase in the mitochondrion and cytosol of *T. brucei*, with both forms being products of a single-copy gene (33) provides a convenient system to examine whether TbiscS2 and TbiscU are required for FeS cluster formation outside of the organelle. Indeed, the enzymatic activity of cytosolic aconitase was markedly decreased upon RNAi induction in both TbiscS2 and TbiscU knock-downs. Control immunoblot and EPR analyses confirmed that the reduction of aconitase activity in both mitochondria and cytosol was due to the lack of FeS clusters.

The analysis of subcellular fractions using specific antibodies raised against recombinant proteins revealed the confinement of TbiscS2 and TbiscU to the mitochondria-rich fractions of *T. brucei*. Accordingly, both proteins possess putative N-terminal presequences required for protein targeting into the mitochondrion. Moreover, TbiscS2 lacks an alternative initiation codon required for synthesizing the cytosolic IscS form identified in humans (21). Although we cannot exclude the possibility that a low level of TbiscS2 and/or TbiscU is present in the cytosol below the detection limit of immunoblot analysis, our experimental data suggest that the trypanosome mitochondrion is essential for the maturation of FeS proteins inside as well as outside the organelle, as was observed in yeast (42, 46). In addition to mitochondria, components of the FeS cluster assembly machinery were recently localized to the mitochondrion-related hydrogenosomes and mitosomes of the parasitic protists *Trichomonas vaginalis* and *Giardia intestinalis*, respectively (14, 15, 47, 48). Based on these findings from evolutionarily distant organisms, it seems that the formation of cellular FeS clusters is a general role of mitochondria in eukaryotic cells and that this role was probably inherited from a premitochondrial endosymbiont (14, 49, 50). Further studies are required to elucidate why the FeS cluster-producing pathway was transferred to another cellular com-

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\(^3\) V. Vilimová, E. Horáková, A. Horváth, J. Lukeš, and J. Tachezy, unpublished data.
partment in humans, a pattern that is currently under debate (11, 24), and if so, at which step of evolution this pattern appeared.

In T. brucei, one of the major differences between the procyclic stage of the insect vector and the blood stage parasitizing mammals is the way they generate energy, in particular the use of the mitochondrion in the process (10, 51). Remarkably, the overall metabolic changes observed in the FeS cluster-impaired cells resulted in a phenotype that mimics the interstitial stage of the organellum. The mitochondrion of the TbiscS2 and TbiscU procyclic knock-downs resembled its counterpart in humans, a pattern that is currently under debate.

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