Recent data suggest that frataxin plays a key role in eukaryote cellular iron metabolism, particularly in mitochondrial heme and iron-sulfur (FeS) cluster biosynthesis. We have now identified a frataxin homologue (T. vaginalis frataxin) from the human parasite Trichomonas vaginalis. Instead of mitochondria, this unicellular eukaryote possesses hydrogenosomes, peculiar organelles that produce hydrogen but nevertheless share common ancestry with mitochondria. T. vaginalis frataxin contains conserved residues implicated in iron binding, and in silico, it is predicted to form a typical α-β sandwich motif. The short N-terminal extension of T. vaginalis frataxin resembles presequences that target proteins to hydrogenosomes, a prediction confirmed by the results of overexpression of T. vaginalis frataxin in T. vaginalis. When expressed in the mitochondria of a frataxin-deficient Saccharomyces cerevisiae strain, T. vaginalis frataxin partially restored defects in heme and FeS cluster biosynthesis. Although components of heme synthesis or heme-containing proteins have not been found in T. vaginalis to date, T. vaginalis frataxin was also shown to interact with S. cerevisiae ferrochelatase by using a Biacore assay. The discovery of conserved iron-metabolizing pathways in mitochondria and hydrogenosomes provides additional evidence not only of their common evolutionary history, but also of the fundamental importance of this pathway for eukaryotes.

In eukaryotes, mitochondrial proteins play vital roles in cellular iron metabolism by inserting iron into two types of prosthetic groups: FeS clusters and heme. While the steps mediating porphyrin synthesis are partitioned between mitochondria and the cytosol, the final insertion of ferrous iron into the porphyrin ring occurs in the mitochondria by the activity of ferrochelatase (7). FeS cluster biosynthesis is catalyzed by a multiprotein machinery (25, 37) with a predominantly mitochondrial localization, although in higher eukaryotes, subpopulations of some components with extramitochondrial localizations were identified (31). The process is initiated by sulfur release from free cysteine by the activity of the cysteine desulfurase IscS (47). A protein-bound persulfide is combined with a still-undefined iron intermediate to form an FeS cluster on the scaffold protein IscU. Both the heme and FeS cluster synthesis pathways rely on an iron donor within the mitochondria to distribute iron, maintain its bioavailability, and reduce its deleterious effects via Fenton chemistry. The nature of such an iron donor remains unclear; however, the small mitochondrial protein frataxin is currently the leading candidate for this function. In humans, loss of frataxin function leads to the neurodegenerative disorder Friedreich’s ataxia, which is manifested on the cellular level by mitochondrial iron accumulation, sensitivity to oxidants, depletion of mitochondrial DNA, impaired respiration, and decreased activities of FeS proteins (6, 13, 23, 45). Because of the difficulties in distinguishing between primary and secondary frataxin-associated phenotypes, the actual biochemical function of frataxin remains unresolved. However, the characterization of the yeast Saccharomyces cerevisiae’s frataxin homologue (Yfh1) suggests several iron-related functions. (i) In vitro studies suggest that frataxin forms large complexes with iron, which may be a mechanism for iron detoxification, reminiscent of the function of the iron storage protein ferritin (14). (ii) The participation of frataxin in FeS cluster assembly was demonstrated by its iron-dependent association with IscU or the IscS/IscU complex (15), where frataxin delivers iron to IscU (46). (iii) Recently, frataxin was shown to protect the FeS cluster of mitochondrial aconitase, functioning as an iron chaperone in the conversion of the inactive [3Fe-4S] form into the active [4Fe-4S] form of the enzyme. (iv) Based upon the observation of reduced cytochrome levels in a Δyfh1 strain, frataxin was also suggested to participate in heme synthesis (23). In these studies, zinc protoporphyrin was found in the affected cells instead of heme, indicating a lack of iron for the insertion step. Frataxin was further shown to physically interact with ferrochelatase in vitro, suggesting its involvement in ferrochelatase-mediated iron chemistry. To date nothing is known about the presence and possible functions of frataxin in eukaryotes, such as Trichomonas, Giardia, and Entamoeba, that lack typical mitochondria. These

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Frataxin, a Conserved Mitochondrial Protein, in the Hydrogenosome of Trichomonas vaginalis

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In eukaryotes, mitochondria play key roles in eukaryote cellular iron metabolism, particularly in mitochondrial heme and iron-sulfur (FeS) cluster assembly. We have now identified a frataxin homologue (T. vaginalis frataxin) from the human parasite Trichomonas vaginalis. Instead of mitochondria, this unicellular eukaryote possesses hydrogenosomes, peculiar organelles that produce hydrogen but nevertheless share common ancestry with mitochondria. T. vaginalis frataxin contains conserved residues implicated in iron binding, and in silico, it is predicted to form a typical α-β sandwich motif. The short N-terminal extension of T. vaginalis frataxin resembles presequences that target proteins to hydrogenosomes, a prediction confirmed by the results of overexpression of T. vaginalis frataxin in T. vaginalis. When expressed in the mitochondria of a frataxin-deficient Saccharomyces cerevisiae strain, T. vaginalis frataxin partially restored defects in heme and FeS cluster biosynthesis. Although components of heme synthesis or heme-containing proteins have not been found in T. vaginalis to date, T. vaginalis frataxin was also shown to interact with S. cerevisiae ferrochelatase by using a Biacore assay. The discovery of conserved iron-metabolizing pathways in mitochondria and hydrogenosomes provides additional evidence not only of their common evolutionary history, but also of the fundamental importance of this pathway for eukaryotes.

In eukaryotes, mitochondrial proteins play vital roles in cellular iron metabolism by inserting iron into two types of prosthetic groups: FeS clusters and heme. While the steps mediating porphyrin synthesis are partitioned between mitochondria and the cytosol, the final insertion of ferrous iron into the porphyrin ring occurs in the mitochondria by the activity of ferrochelatase (7). FeS cluster biosynthesis is catalyzed by a multiprotein machinery (25, 37) with a predominantly mitochondrial localization, although in higher eukaryotes, subpopulations of some components with extramitochondrial localizations were identified (31). The process is initiated by sulfur release from free cysteine by the activity of the cysteine desulfurase IscS (47). A protein-bound persulfide is combined with a still-undefined iron intermediate to form an FeS cluster on the scaffold protein IscU. Both the heme and FeS cluster synthesis pathways rely on an iron donor within the mitochondria to distribute iron, maintain its bioavailability, and reduce its deleterious effects via Fenton chemistry. The nature of such an iron donor remains unclear; however, the small mitochondrial protein frataxin is currently the leading candidate for this function. In humans, loss of frataxin function leads to the neurodegenerative disorder Friedreich’s ataxia, which is manifested on the cellular level by mitochondrial iron accumulation, sensitivity to oxidants, depletion of mitochondrial DNA, impaired respiration, and decreased activities of FeS proteins (6, 13, 23, 45). Because of the difficulties in distinguishing between primary and secondary frataxin-associated phenotypes, the actual biochemical function of frataxin remains unresolved. However, the characterization of the yeast Saccharomyces cerevisiae’s frataxin homologue (Yfh1) suggests several iron-related functions. (i) In vitro studies suggest that frataxin forms large complexes with iron, which may be a mechanism for iron detoxification, reminiscent of the function of the iron storage protein ferritin (14). (ii) The participation of frataxin in FeS cluster assembly was demonstrated by its iron-dependent association with IscU or the IscS/IscU complex (15), where frataxin delivers iron to IscU (46). (iii) Recently, frataxin was shown to protect the FeS cluster of mitochondrial aconitase, functioning as an iron chaperone in the conversion of the inactive [3Fe-4S] form into the active [4Fe-4S] form of the enzyme. (iv) Based upon the observation of reduced cytochrome levels in a Δyfh1 strain, frataxin was also suggested to participate in heme synthesis (23). In these studies, zinc protoporphyrin was found in the affected cells instead of heme, indicating a lack of iron for the insertion step. Frataxin was further shown to physically interact with ferrochelatase in vitro, suggesting its involvement in ferrochelatase-mediated iron chemistry. To date nothing is known about the presence and possible functions of frataxin in eukaryotes, such as Trichomonas, Giardia, and Entamoeba, that lack typical mitochondria. These
unicellular organisms inhabit oxygen-poor environments, and their energy metabolism relies upon the glycolytic breakdown of glucose within the cytosol. While in *Giardia* and *Entamoeba*, pyruvate is further oxidized by cytosolic pathways, in *Trichomonas vaginalis*, pyruvate breakdown takes place in double membrane-bound organelles called hydrogenosomes. The key hydrogenosomal enzymes (PFO and hydrogenase), as well as the components of the hydrogenosomal electron transport chain (ferredoxin and the NADH dehydrogenase complex), are FeS proteins. The FeS clusters of these proteins are assembled within hydrogenosomes by a mitochondrial-type machinery involving a hydrogenosomal homologue of IscS (38, 39). In this paper, we describe a homologue of frataxin in *T. vaginalis*. Based upon its hydrogenosomal localization, its biochemistry, and its ability to complement the function of a yeast mitochondrial mutant, we conclude that frataxin in hydrogenosomes is functionally orthologous to the mitochondrial protein implicated in cellular iron metabolism.

**MATERIALS AND METHODS**

**Cultivation and cell fractionation.** *T. vaginalis* strain T1 was maintained in Trypticase-yeast extract-maltose medium with 10% heat-inactivated horse serum at 37°C. Cytosolic and hydrogenosomal fractions were prepared as described in reference 38.

**Cloning and sequence analysis.** A sequence encoding a nearly complete frataxin homologue was identified in a *T. vaginalis* G3 expressed sequence tag library and used to isolate a full-length sequence from a *T. vaginalis* T1 ZAPII genomic library clone. The probe for library screening was generated by PCR using the specific primers 5'-CTTCTGTAAACAAAC-3' and 5'-CTTCTGTAAACAAAC-3'. The PCR products were labeled using a random primer DNA labeling system (Invitrogen). Positive clones were sequenced and compared with bacterial and mitochondrial frataxin protein sequences in GenBank. The sequences were aligned using ClustalX (40), and the alignment was refined manually.

**RNA transcription.** The synthesis of nascent mRNA was assessed by polycisthelin-permeabilized cells (43). The following primer pairs were used in PCR amplifications: frataxin, 5'-ATGTTAAGCAGGATT-3' and 5'-TTAGAACAGGAAAGC-3'; Fos-1, 5'-GAGTGGCAGGACCG-3' and 5'-ACCTGGCATCCTCAAGAAGG-3'; and 5'-AACAGGTTGAACG-3'; hydrogenosomal malic enzyme, 5'-AGAACACATGAGG-3'. The purity of the final preparation was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a Coomassie-stained 14% gel.

The direct interaction between ferrochelatase and *T. vaginalis* frataxin was monitored by using a real-time bioluminescence assay, Biacore 2000, based on plasmon surface resonance measurements. *T. vaginalis* frataxin (200 resonance units [RU]) was immobilized on research grade CM sensor chips (Biacore) using a standard amine-coupling procedure. Control experiments were run using bovine serum albumin (200 RU) and blank flow cells (activated carboxyl groups reacted with excess ethanolamine). The circulating ferrochelatase was at a concentration of 1 x 10⁻⁷ M.

**Complementation assay.** The complete open reading frame of the *T. vaginalis* frataxin gene was amplified and cloned into the pEMBlyex4i vector using the NdeI and Xhol sites. The mature version of the *T. vaginalis* frataxin gene was cloned in-frame behind the CoxIV mitochondrial-targeting sequence within the pEMBlyex4i vector using the XbaI and Xhol restriction sites. The vectors were linearized with Stul in the URA3 gene and used for transformation of an *S. cerevisiae* YH11 shuffle strain (17). The transformants were selected for uracil prototrophy, and after removal of the covering plasmid containing YFHI, LEU2, and CYH2 by counterselection on YPAD (yeast extract-peptone-dextrose plus adenine) with 10 µg/ml cycloheximide, transformants were tested for growth under inducing conditions (1% yeast extract, 2% peptone, 0.01% adenine, 2% raffinose, and 0.5% galactose) for the GAL10 promoter of the integrated pEMBlyex4i vector.

**Other methods.** Aconitase and malate dehydrogenase activities were measured spectrophotometrically in mitochondrial extracts (5, 28, 34). The low-temperature (~191°C) spectra of whole cells were recorded as described previously (30).

**RESULTS**

**Analysis of the *T. vaginalis* frataxin gene.** The complete sequence of the frataxin gene from *T. vaginalis* was obtained from a *T. vaginalis* genomic DNA library clone. The isolated clone contained a single putative open reading frame without intron-like sequences and coded for a protein of 121 amino acids in length. The predicted molecular mass and isoelectric point for the putative *T. vaginalis* frataxin were 13.8 kDa and 4.9, respectively. Similar acidic pI values have been reported for all frataxin homologues, reflecting the high content of Asp and Glu residues in the protein.

BLASTP searches yielded the highest scores with eukaryotic and eubacterial (*CyaY*) frataxin homologues. No frataxin homologue has been identified in any archaeabacterial species so far. The protein sequence of *T. vaginalis* frataxin was compared with those of its homologues from eubacteria and eukaryotes (Fig. 1). In eukaryotes, the sequence of the mature protein is typically preceded by a variable N-terminal region that carries a mitochondrial-targeting signal sequence. The structures of human, yeast, and bacterial frataxin have been solved recently (10, 12, 18), showing that these homologues are defined by an α-β sandwich motif, with two N-terminal and C-terminal α-helices forming a helical plane above five antiparallel β-strands forming a β-sheet plane. While the β-sheet plane is rather neutral in charge, the helical plane and the H1-S1 interface contain conserved and exposed acidic residues (18). Possible iron-binding residues were identified in this region and are conserved in the *T. vaginalis* homologue (Fig. 1), suggesting that it may have similar iron-binding properties. Using the SWISS-MODEL program (http://swissmodel.expasy.org/SWISS-MODEL.html) (35), the theoretical structure of *T. vaginalis* frataxin was derived, using human frataxin as a template. The model of *T. vaginalis* frataxin revealed that the predicted polypeptide chain could form the characteristic frataxin α-β sandwich motif (Fig. 2). No structure was assigned to three loops of the polypeptide chain due to the lack of sequence similarity between homologues in these regions.
The analysis of the 5′ upstream region preceding the T. vaginalis frataxin gene open reading frame did not reveal the presence of a conserved initiator (Inr) element (Fig. 3A). This element, consisting of TCA/TTATA as a consensus sequence, determines the transcription start site (at A/TTAT position) and has been found in all T. vaginalis genes examined so far (26). The mRNAs of T. vaginalis possess unusual short 5′ untranscribed regions of about 10 to 17 bp before the translation start, and the modification of the Inr consensus sequence results in the generation of mRNAs with inaccurate transcription start sites, thereby interfering with gene expression (26). The transcription of the frataxin gene was therefore verified by monitoring mRNA synthesis in permeabilized cells (Fig. 3B).

In S. cerevisiae, frataxin expression is stimulated by iron (32, 33), and iron has also been shown to dramatically regulate the metabolism of trichomonads (42). During iron deficiency, the expression of key hydrogenosomal enzymes (e.g., PFO and hydrogenase) dramatically decreases and overall glucose metabolism is shifted towards cytosolic glycolytic breakdown. The synthesis of mRNA under iron-rich and iron-restricted conditions was monitored to examine the possible effects of iron availability on the transcription of the T. vaginalis frataxin gene. Whereas the transcription of pfo and tvh-47 was decreased under iron-restricted conditions, T. vaginalis frataxin gene transcription increased about threefold (Fig. 3B), in contrast to the data obtained from yeast and human cells.

Cellular localization of T. vaginalis frataxin. In eukaryotes, frataxin is localized primarily in the mitochondria, although a small amount of extramitochondrial frataxin has recently been

FIG. 1. Multiple protein sequence alignment of eukaryotic and eubacterial frataxin homologues. The proteins were aligned using the ClustalX program (40). The black shading indicates where identical amino acids are conserved; the gray shading shows similar amino acids, with the threshold set to 50%. The mitochondrial-targeting and putative hydrogenosomal sequences are in italics and underlined. In the human frataxin sequence, 25 N-terminal amino acids were omitted from the alignment. Asterisks denote identical/similar residues implicated in iron binding, which are conserved in T. vaginalis frataxin. Secondary-structure elements of E. coli CyaY are indicated above the sequence alignment (according to reference 10).

FIG. 2. Model of T. vaginalis frataxin. The three-dimensional structure of T. vaginalis frataxin was deduced by using the theoretically translated frataxin sequence (B). The previously solved crystal structure of human frataxin (PDB accession number 1EKG) was used as a template (A). The primary sequence alignment was generated by using ClustalW (http://www.ebi.ac.uk/clustalw), edited manually and submitted to SWISS-MODEL (http://swissmodel.expasy.org//SWISS-MODEL.html) (35). The unmodeled regions of T. vaginalis frataxin are shown in red.

FIG. 3. Transcription of T. vaginalis frataxin gene. (A) Conserved motifs of putative initiator elements (in uppercase) in the sequences of 5′ regions preceding the open reading frame of the T. vaginalis α subunit of succinyl-coenzyme A synthase (α-SCS), ferredoxin, and frataxin. The nucleotides corresponding to the start codons are underlined. (B) Synthesis of nascent RNA. Cells maintained under iron-rich (Fe++) or iron-restricted (Fe++) conditions were permeabilized with lysocinolcin and incubated with buffer containing [32P]UTP. Total RNA was isolated and hybridized to specific DNA probes. PFO, pyruvate:ferredoxin oxidoreductase; Tvh-47, subunit of hydrogenosomal NADH dehydrogenase complex; β-tub, β-tubulin.
described in human cell lines (11). Efficient delivery into the organelle is ensured by the presence of an N-terminal targeting sequence that is cleaved upon protein translocation. As previously shown (39), the prediction software designed to identify mitochondrial-targeting signals can sometimes be successfully used for the detection of signals on hydrogenosomal proteins. PSORT II (http://psort.ims.u-tokyo.ac.jp) was used to predict the localization of *T. vaginalis* frataxin. A mitochondrial/hydrogenosomal localization was predicted with 45% confidence and was further supported by the identification of a putative cleavage site for the organellar processing peptidase (SRS/IM) with a characteristic arginine at position relative to the cleavage site. We overexpressed *T. vaginalis* frataxin with a C-terminal HA tag in trichomonads, and Western blot analysis of the cellular fractions showed the presence of recombinant *T. vaginalis* frataxin in the cell homogenate and purified hydrogenosomes (Fig. 4A). The estimated molecular mass of about 16 kDa corresponded to the theoretical frataxin size, including the double HA tag.

By means of immunofluorescence microscopy, the HA-tagged version of *T. vaginalis* frataxin was localized within numerous organelles surrounding the nucleus and main cytoskeletal structures (Fig. 4B). These organelles correspond to hydrogenosomes, as demonstrated by the colocalization of the HA tag signal with hydrogenosomal malic enzyme in a double-labeling experiment.

**Complementation of yeast frataxin mutants.** Previous studies reported the defective respiration, unstable mitochondrial DNA, and hypersensitivity to oxidative stress that follow inactivation of the yeast frataxin homologue (Yfh1) (6, 20). The impairment of mitochondrial iron metabolism, particularly FeS cluster and heme synthesis, was suggested to be the primary cause for this complex phenotype (48). To study the function of *T. vaginalis* frataxin, we tested its ability to complement the Yfh1 function in yeast.

One of the difficulties when working with Δ*yfh1* cells is that suppressor mutations occur with high frequency (23). For this reason, we used a Yfh1 shuffle strain (17), in which a covering plasmid bearing the wild copy of *YFH1* can be ejected by growing the cells with cycloheximide. The complete *T. vaginalis* frataxin and chimeric CoxIV-*T. vaginalis* frataxin product, comprising the CoxIV mitochondrial-targeting sequence and the *T. vaginalis* frataxin sequence minus its hydrogenosomal-targeting sequence, were each used to transform the Yfh1 shuffle strain. The wild-type *YFH1* open reading frame and no open reading frame were used as the positive and negative controls, respectively. The expression of all constructs was under the control of the Gal promoter. The transformants were plated onto yeast extract-peptone-dextrose agar plates and then transferred to cycloheximide-containing YPAGal (yeast extract-peptone-adenine-galactose) plates to eject the covering plasmid. Significant growth differences were observed (Fig. 5).
FIG. 5. Complementation of a yeast mutant deficient in frataxin (Δyfh1). The gene for *T. vaginalis* frataxin (Tvfrataxin) and a chimeric construct containing genes for the mitochondrial-targeting sequence of CoxIV and for *T. vaginalis* frataxin (CoxIV-Tvfrataxin) truncated at the N terminus to remove the putative hydrogenosomal-targeting signal were integrated into the genome of the Δyfh1 mutant under the control of the Gal1 promoter mutant by using the shuffle strategy. Wild-type YFH1 and the empty vector (Δyfh1) served as the positive and negative controls, respectively. Serial 10-fold dilutions of 10^6 cells are shown.

The Gal-*T. vaginalis* frataxin transformant grew poorly under these conditions, recapitulating the strong growth defect of the Δyfh1 mutant. However, there was a partial restoration of growth in the cells with the Gal-CoxIV-*T. vaginalis* frataxin construct. This result indicates that *T. vaginalis* frataxin can partially complement the function of Yfh1. The complementation requires the presence of the N-terminal targeting sequence from CoxIV to enable the translocation of the protein into the mitochondria. Analysis of yeast cellular fractions (data not shown) revealed that *T. vaginalis* frataxin possessing only the hydrogenosomal-targeting sequence did indeed remain in the cytosol and was not translocated into the mitochondria.

**Ability of *T. vaginalis* frataxin to restore FeS cluster and heme synthesis.** To examine the molecular basis for the partial restoration of the growth of the Δyfh1 mutant by *T. vaginalis* frataxin, we investigated the potential of *T. vaginalis* frataxin to restore FeS cluster and heme synthesis. The activity of the mitochondrial FeS protein aconitase was measured in yeast expressing Yfh1 and *T. vaginalis* frataxin (Fig. 6). Comparison of the aconitase activity of yeast containing the Gal-CoxIV-*T. vaginalis* frataxin construct with the aconitase activities of positive (Gal-Yfh1) and negative controls showed that Gal-CoxIV-*T. vaginalis* frataxin was able to restore about 50% of the specific activity conferred by Gal-Yfh1. There was no restoration of aconitase activity by Gal-*T. vaginalis* frataxin alone, which is consistent with the inability of this construct to improve the growth of the Δyfh1 mutant. The partial complementation of aconitase activity by CoxIV-*T. vaginalis* frataxin suggests that the trichomonad homologue, when present in the mitochondria, may cooperate with yeast IscU and participate in FeS cluster formation. Malate dehydrogenase, the activity of which is not dependent on FeS cluster formation, was not affected in the Gal-*T. vaginalis* frataxin and Δyfh1 mutants (Fig. 5).

Low-temperature spectra of whole cells were recorded to evaluate the levels of heme synthesis. Colonies of the Δyfh1 mutant are known to be depigmented as the result of general heme deficiency. In our experiment, the Gal-CoxIV-*T. vaginalis* frataxin strain showed increased levels of cytochrome (b and c) signals in comparison with the levels in the Δyfh1 mutant (Fig. 7). Interestingly, a clear signal from zinc protoporphyrin was observed in the complemented strain. The accumulation of zinc protoporphyrin, which is a feature of the Δyfh1 phenotype, reflects inadequate iron or unavailable iron (23). The Gal-CoxIV-*T. vaginalis* frataxin strain partially complemented the deletion, suggesting that CoxIV-*T. vaginalis* frataxin is able to interact at low efficiency in vivo with yeast ferrochelatase. *T. vaginalis* frataxin and ferrochelatase interact in vitro. To investigate the interaction of *T. vaginalis* frataxin with its pre-

![Graph showing aconitase activity](image)

**FIG. 6.** Aconitase activity in the complemented yeast strain. The levels of specific activity of the FeS enzyme aconitase were determined in mitochondrial extracts of the Δyfh1 (delta-yfh1) mutant transformed with *T. vaginalis* frataxin (Tvfrataxin), a chimeric construct containing the mitochondrial-targeting sequence of CoxIV (CoxIV-Tvfrataxin), and a wild copy of the gene (YFH1). The activity of the non-FeS enzyme malate dehydrogenase (MDH) was used as a control. Bars indicate standard deviations; n = 6.

![Graph showing cytochrome composition](image)

**FIG. 7.** Cytochrome composition in the complemented yeast strain. Low-temperature spectra of whole cells of the Yfh1 shuffle strain with integrated Gal-YFH1 (1), Gal-CoxIV-*T. vaginalis* frataxin (2), and vector alone (3). In cells complemented with *T. vaginalis* frataxin, zinc protoporphyrin accumulated, indicating inefficient iron administration of *T. vaginalis* frataxin to ferrochelatase.
dicted interacting partners, we used a real-time biomolecular interaction analyzer, Biacore 2000. A specific interaction between *T. vaginalis* frataxin and ferrochelatase was measured when ferrochelatase was used as the analyte (Fig. 8), while no interaction was found with bovine serum albumin as a negative control. The dissociation constant was estimated as $K_D = 7e^{-9}$ by fitting to a simple Langmuir model.

**DISCUSSION**

Homologues of frataxin are present in organisms ranging from eubacteria to mammalian cells but are absent in archaeabacteria. They are highly conserved proteins with no homology identified outside the frataxin family. In *S. cerevisiae* and humans, frataxin is believed to have an important role in mitochondrial iron metabolism (15, 23). The current data demonstrate that hydrogenosomes of *T. vaginalis* also possess functional frataxin and suggest that this protein is likely to participate, either directly or indirectly, in the synthesis of FeS clusters within hydrogenosomes.

The frataxin sequence identified in *T. vaginalis* possesses features typical of the protein family as found for the human and yeast homologues. Conserved acidic residues implicated in iron binding are found at the N terminus of *T. vaginalis* frataxin, suggesting that the protein may possess similar properties in mobilizing iron for its interacting partners (2, 18). For Yfh1, it was shown that its iron-binding residues significantly overlap with those involved in ferrochelatase binding, arguing for iron transfer from frataxin to ferrochelatase (18). Indeed, as demonstrated by the Biacore assay, *T. vaginalis* frataxin is able to interact in vitro with yeast ferrochelatase, the enzyme responsible for catalyzing the insertion of iron into the protoporphyrin ring during mitochondrial heme biosynthesis. Consistently, *T. vaginalis* frataxin is able to partially restore defective heme synthesis in a Δyfh1 mutant. However, a fraction of protoporphyrin molecules contained zinc instead of iron in CoxIV-*T. vaginalis* frataxin yeast cells, likely reflecting an inefficient delivery of iron by the heterologous frataxin. Hypothetically, this might be due to the specificity of *T. vaginalis* frataxin for different natural partners, since *T. vaginalis* lacks cytochromes and no components of the heme synthesis pathway, including the homologue of ferrochelatase, have been found in the recently completed *T. vaginalis* genome (9).

Besides an involvement in heme synthesis, the yeast frataxin was shown to physically interact with IscU, a central component of FeS cluster assembly (15). IscU serves as a scaffold molecule for building an FeS cluster intermediate in participation with IscS that provides the required sulfur from free cysteine (3, 47). The transient FeS cluster on IscU is then transferred to the target apoprotein. The detection of frataxin in complex with the IscU/IscS pair suggested that frataxin also participates in FeS cluster assembly—possibly as an iron donor (15). FeS cluster synthesis has already been shown to occur in *T. vaginalis* hydrogenosomes via an IscS-mediated mitochondrial-type system (38). Although IscU has not been characterized in *T. vaginalis*, an IscU homologue is present in the *T. vaginalis* genome (9). Moreover, the homologue possesses a putative hydrogenosomal-targeting sequence, which indicates its potential colocalization with IscS and frataxin within the hydrogenosomal compartment. Thus, it is plausible that an IscS/IscU/frataxin complex is formed in *T. vaginalis* hydrogenosomes and participates in FeS cluster synthesis.

The stimulation of frataxin transcription during iron deficiency may indicate a common mode of regulation with IscS, the transcription of which was also shown to be upregulated in iron deficiency (38). This contrasts with the transcription of key hydrogenosomal proteins involved in energy metabolism and the transport of electrons, which are often FeS proteins and whose transcription is dramatically reduced in the absence of iron (42). The reason for frataxin and IscS upregulation might be the increased cellular demand for new FeS cluster synthesis, although more experimental data are necessary to test this hypothesis. Interestingly, in the yeasts *S. cerevisiae* and *C. albicans*, frataxin transcription was shown to be strongly induced by iron (32, 33). This finding is consistent with the proposed role of frataxin as an iron storage molecule. For example, yeast
Yfh1 was shown to self assemble in an iron-dependent manner and to sequester iron in vitro, and it was proposed that by this mechanism, reminiscent of the function of mammalian ferritin, frataxin keeps iron bioavailable and nontoxic (14, 29). Moreover, the overexpression of newly identified mitochondrial ferritin (8) was able to repair the phenotype of a frataxin-deficient strain, supporting a partial redundancy of these two iron-bind- ing proteins. However, the in vivo relevance of iron-dependent polymerization of Yfh1 was put in question by the ability of modified nonpolymerizing Yfh1 to fully restore the phenotype of a Δyfh1 mutant (5).

Recent discoveries in FeS cluster assembly in eukaryotes have underlined the essential role of mitochondria in this process (24, 25). Defects in the expression of components involved in FeS cluster synthesis often have dramatic or even lethal consequences for the whole organism. Consequently, it has been suggested that FeS cluster synthesis might be the critical indispensable function of mitochondria and of the mitochondrial homologues, hydrogenosomes and mitosomes, recently identified in some microaerophilic and parasitic unicellular eukaryotes (22, 38, 41). The finding of a frataxin homologue in identified in some microaerophilic and parasitic unicellular drial homologues, hydrogenosomes and mitosomes, recently indispensable function of mitochondria and of the mitochon-

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clusters (4, 44). Thus, the situation is more complicated

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