Giardia mitosomes and trichomonad hydrogenosomes share a common mode of protein targeting

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Mitochondria are archetypal organelles of endosymbiotic origin in eukaryotic cells. Some unicellular eukaryotes (protists) were considered to be primarily amitochondrial organisms that diverged from the eukaryotic lineage before the acquisition of the premitochondrial endosymbiont, but their amitochondrial status was recently challenged by the discovery of mitochondria-like double membrane-bound organelles called mitosomes. Here, we report that proteins targeted into mitosomes of *Giardia intestinalis* have targeting signals necessary and sufficient to be recognized by the mitosomal protein import machinery. Expression of these mitosomal proteins in *Trichomonas vaginalis* results in targeting to hydrogenosomes, a hydrogen-producing form of mitochondria. We identify, in *Giardia* and *Trichomonas*, proteins related to the component of the translocase in the inner membrane from mitochondria and the processing peptidase. A shared mode of protein targeting supports the hypothesis that mitosomes, hydrogenosomes, and mitochondria represent different forms of the same fundamental organelle having evolved under distinct selection pressures.

biogenesis | FeS cluster assembly | Pam18 | matrix-located processing peptidase | ferredoxin

Mitosomes are double-membrane bound organelles found in some unicellular eukaryotes, including *Entamoeba histolytica* (1, 2) and microsporidians such as *Trachipleistophora hominis* (3). The name "mitosome" (synonym: crypton) was proposed to indicate that the organelles are highly reduced (cryptic) mitochondria (1). More recently, mitosomes were identified in the human intestinal parasite *Giardia intestinalis* (4), which has often been considered to be among the earliest branching eukaryotes (5, 6). The apparent lack of mitochondria in *Giardia* had led to the hypothesis that *Giardia* separated from other eukaryotes before the acquisition of mitochondria (7). The presence of mitosomes in *Giardia* provides evidence that even if *Giardia* really is an early branching eukaryote, it nevertheless split from other eukaryotes after the mitochondrial endosymbiosis event (4). This view is further supported by identification of several genes of putative mitochondrial origin on the *Giardia* genome (8, 9).

A key piece of evidence for identifying the mitosomes in *Giardia* was the discovery that they contain components of the protein machinery responsible for iron sulfur cluster assembly (10). Cysteine desulfurase (IscS) and a scaffold protein (IscU) carry out the crucial steps in biosynthesis of Fe-S centers. In eukaryotes, this process takes place exclusively in double membrane-bound organelles including mitochondria (11), hydrogenosomes (12), and chloroplasts (13). Phylogenetic analyses placed the *Giardia* IscS (GiiscS) within the mitochondrion/hydrogenosome clade (10, 14). In addition, GiiscS and *Giardia* scaffold protein (GiiscU) colocalized inside vesicles surrounded by a double membrane and high-speed cellular fractions of *Giardia* catalyzed reconstitution of FeS clusters in an apoprotein lacking FeS moieties (4). Based on these data, it has been proposed that the GiiscS- and GiiscU-containing vesicles are highly reduced mitochondrial homologues or mitosomes.

The presence of a common type of FeS assembly machinery in *Giardia* mitosomes, trichomonad hydrogenosomes, and mitochondria argues for a common evolutionary history of these organelles (4); however, it does not refute contentions that these organelles each arose independently from related species of bacterial endosymbionts (15). One problem is the absence of knowledge concerning the biogenesis of the mitosomes, the evidence that provided strong arguments for a common progenitor of hydrogenosomes and mitochondria (16, 17). Proteins targeted into the mitochondria are synthesized in cytosol with an N-terminal extension for protein targeting; however, many have internal targeting signals. Both sorts of targeting information are recognized by the outer (TOM) and inner (TIM) membrane translocases (18, 19). The mitochondrial matrix proteins are further translocated through the TIM23 complex, with energy supplied by a PAM complex. The PAM complex includes an integral membrane protein with a J domain referred to either as Pam18 (20) or Tim14 (21). After translocation, N-terminal presequences are then cleaved by a matrix-located processing peptidase (MPP) (22). Proteins targeted to hydrogenosomes have N-terminal extensions that carry targeting information (23). Interestingly, initial work on the proteins assembling Fe-S centers in *Giardia* showed that two mitosomal proteins, GiiscU (4) and [2Fe2S] ferredoxin (24), have also predicted N-terminal extensions, whereas such an extension was absent in GiiscS (4).

To provide insight into the biogenesis of *Giardia* mitosomes, we investigated and compared targeting of GiiscS, GiiscU, and [2Fe2S] ferredoxin to *Giardia* mitosomes and to hydrogenosomes in *Trichomonas vaginalis*. We show that mitosomes and hydrogenosomes share a common mode of protein targeting that, like protein import into mitochondria, can make use of N-terminal or internal targeting signals. Initial sequence analysis and cell localization studies suggests that *Giardia* and *Trichomonas* have protein import machinery that shares common components with the protein import machinery of mitochondria and mitochondria-like processing peptidases.

Materials and Methods

Cell Cultivation. *G. intestinalis* strain WB (American Type Culture Collection) was grown in TYI-S-33 medium supplemented with antibiotics (25). *T. vaginalis* strain T1 (kindly provided by P. J. Johnson, University of California, Los Angeles) was maintained in TYM medium (26). *Saccharomyces cerevisiae* strain YPH499 was grown in a rich medium as described in ref. 12.

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Abbreviations: GiiscS, *Giardia* cysteine desulfurase; GiiscU, *Giardia* scaffold protein; MPP, matrix-located processing peptidase.

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Selectable Transformation of *G. intestinalis* and *T. vaginalis*. The plasmid pONDRA-HA was constructed by modifying pRANneoGDHluc (27). The luc gene was replaced with the HA tag cassette from TagVag vector (28), and the 5′ UTR of GDH was modified for further cloning. The *giiscu*, *gifdx*, Δ*giiscu*, Δ*gifdx*, and *giα-tub* genes were amplified by PCR from genomic DNA and introduced into plasmids for transformation as described in ref. 27. All primers used in this study are described in supporting information, which is published as supporting information on the PNAS web site. For *T. vaginalis* transformation, *Giardia* genes were subcloned into the plasmid TagVag (28). Cells were transformed and selected as described in ref. 12. Iterative BLAST searches were used to identify the Pam18 orthologous sequences from *G. intestinalis* (protein accession no. EAA37663) and *T. vaginalis* (orf 95394.m00357) (29). BLAST searches of GIARDIADB for members of the M16 protease family revealed Giβ MPP (EAA39560). The *tvpam18*, *gipam18*, and *tvβmpp* genes were amplified, cloned, and expressed in *Giardia* and *Trichomonas* as above.

Immunofluorescent Microscopy. Mitosomal proteins were stained in fixed *G. intestinalis* and *T. vaginalis* cells with mouse α-HA mAb (12). In double-labeling experiments, *G. intestinalis* clathrin heavy chain, disulfide isomerase, and GiiscU were detected with rabbit polyclonal Abs (A. Hehl, University of Zürich, Zürich; ref. 4). Hydrogenosomal malic enzyme was detected by rabbit polyclonal Ab (30). Details are given in supporting information.

Preparation of Subcellular Fractions. *Giardia* subcellular fractions were obtained by differential and sucrose gradient centrifugation of the cell homogenate as detailed in supporting information. Percoll-purified hydrogenosomes and cytosol of *T. vaginalis* were prepared as described in ref. 12. Mitochondria were isolated from the *S. cerevisiae* strain YPH499 as described in ref. 31. To remove proteins not imported into the organelles, hydrogenosomes were incubated 60 min with 200 μg/ml trypsin in ST buffer (250 mM sucrose/0.5 mM KCl/10 mM Tris·HCl, pH 7.2) at 37°C and washed twice with 5 mg/ml soybean trypsin inhibitor in ST buffer.

Mitosome-rich fractions were processed for electron microscopy with a modified method of Tokuyasu (32). Ultrathin frozen sections were labeled with mouse α-HA mAb and 10 nm gold-labeled goat α-mouse Ab and observed in a Jeol 1010 electron microscope, as detailed in supporting information.

Protein Processing Assay. GiiscU and ΔGiiscU were cloned into pSP64T (Promega). The constructs were incubated with TNT Quick Coupled Transcription/Translation System (Promega) according to the manufacturer's protocol. Synthesized proteins were precipitated by 60% ammonium sulfate (wt/vol in water), and the resulting precipitate dissolved in buffer (20 mM Tris/1 mM KCl/10 mM $MgCl_2$/0.5% Triton). Organelles (100 μg of protein) were mixed with ^{35}S-labeled protein in the same buffer. Mitochondrial and hydrogenosomal samples were incubated for various times at 30°C and 37°C, and samples were analyzed by SDS/PAGE and autoradiography.

GiiscU Expression and Determination of Processing Site. GiiscU was expressed in *E. coli* by using pQE30 vector (Qiagen) and was affinity purified under native conditions (Qiagen). Protein (≈150 μg) was incubated for 60 min in 10 mM Hepes (pH 7.5)/0.1 mM $MnCl_2$/0.5 mM DTT with 4 μg of recombinant rat MPP (kindly provided by J. Adamec, Academy of Sciences, Prague, Czech Republic) (33). The reaction was inhibited by addition of 10 mM EDTA, and samples were separated on SDS/PAGE gels, blotted to poly(vinylidene difluoride) membrane and stained with Coomassie brilliant blue. Selected protein bands were subjected to N-terminal protein sequencing by Edman degradation.

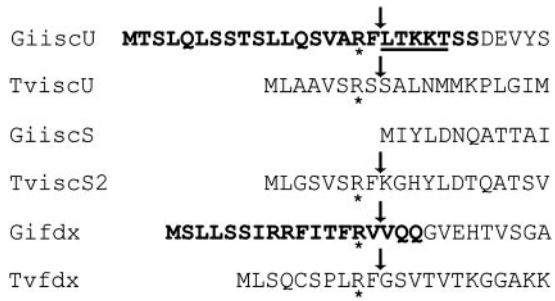

Fig. 1. N-terminal regions of *Giardia* and *Trichomonas* IscU, IscS, and [2Fe-2S] ferredoxins. MITOPROT (http://ihg.gsf.de/ihg/mitoprot.html) predicted targeting sequences are highlighted in bold. PSORT II (http://psort.nibb.ac.jp) cleavage sites (arrows) are shown, and arginines (at position −2 relative to the cleavage site) highlighted by asterisks. N-terminal amino acid sequences determined in GiiscU, retrieved from *Giardia*, or processed *in vitro* by recombinant rat MPP are underlined.

The HA-tagged GiiscU was immunoprecipitated from *G. intestinalis* transformants by using proteinA Sepharose (Sigma), coupled with α-HA mAb adopting the method from ref. 34. Details are given in supporting information.

Results

Targeting of GiiscU, GiiscS, and Gifdx into Mitosomes. *GiiscU*, *Gifdx*, *GiiscS*, and the truncated forms (Δ*giiscU* and Δ*gifdx*) lacking 26 and 18 aa of predicted N-terminal extensions (Fig. 1), respectively, were overexpressed in *Giardia* and *Trichomonas* with a C-terminal HA tag (27, 28). The products of *giiscS*, *giiscU*, and *gifdx* were found in a number of vesicles (30 ± 6 per cell) with a distribution characteristic of mitosomes (4): between the two *Giardia* nuclei in close proximity to the basal bodies and in the lateral and posterior parts of the cell (Fig. 2*A*). Tagged GiiscS colocalized with native GiiscU in double-labeling experiments (data not shown). These vesicles were clearly distinct from the endoplasmic reticulum and peripheral vesicles beneath the plasma membrane (Fig. 2*B*). Subsequently, the mitosome containing fraction from *giiscU* transformants were purified from the homogenate by differential and gradient centrifugation. Immunoelectron microscopy revealed the presence of tagged GiiscU within organelles of ≈184 × 140 nm in diameter, and surrounded by two membranes (Fig. 2*D*). These features indicate that all three proteins were translocated into *Giardia* mitosomes (4).

The N-terminal extensions predicted for Gifdx and GiiscU are necessary for targeting the proteins to mitosomes: weak labeling of mitosomes was observed in cells expressing Δ*giiscU* that lacks the 26-residue N-terminal sequence, and no organellar labeling was observed in the cells expressing Δ*Gifdx* lacking its 18-residue extension (Fig. 2*A*). The targeting function of these N-terminal leader sequences was confirmed by Western blot analysis of the cellular fractions (Fig. 2*C*), with GiiscU and Gifdx present exclusively in the mitosome-rich fraction. By contrast, the majority of ΔGiiscU was found in the cytosol, and no organellar signal was detected for ΔGifdx, although it did not accumulate within the cytosol either. To be certain that ΔGiFdx was expressed, we compared mRNA levels of *gifdx* and Δ*gifdx* in corresponding transformants. No difference in *gifdx* and Δ*gifdx* transcription was found (supporting information). Thus, failure of ΔGifdx to be targeted to mitosomes likely results in degradation of the apoprotein by proteolysis, as previously reported for the apoform of Leu1p in yeast (35).

N-Terminal Targeting Sequence-Independent Import of GiiscS. No N-terminal targeting sequence was predicted for GiiscS. To

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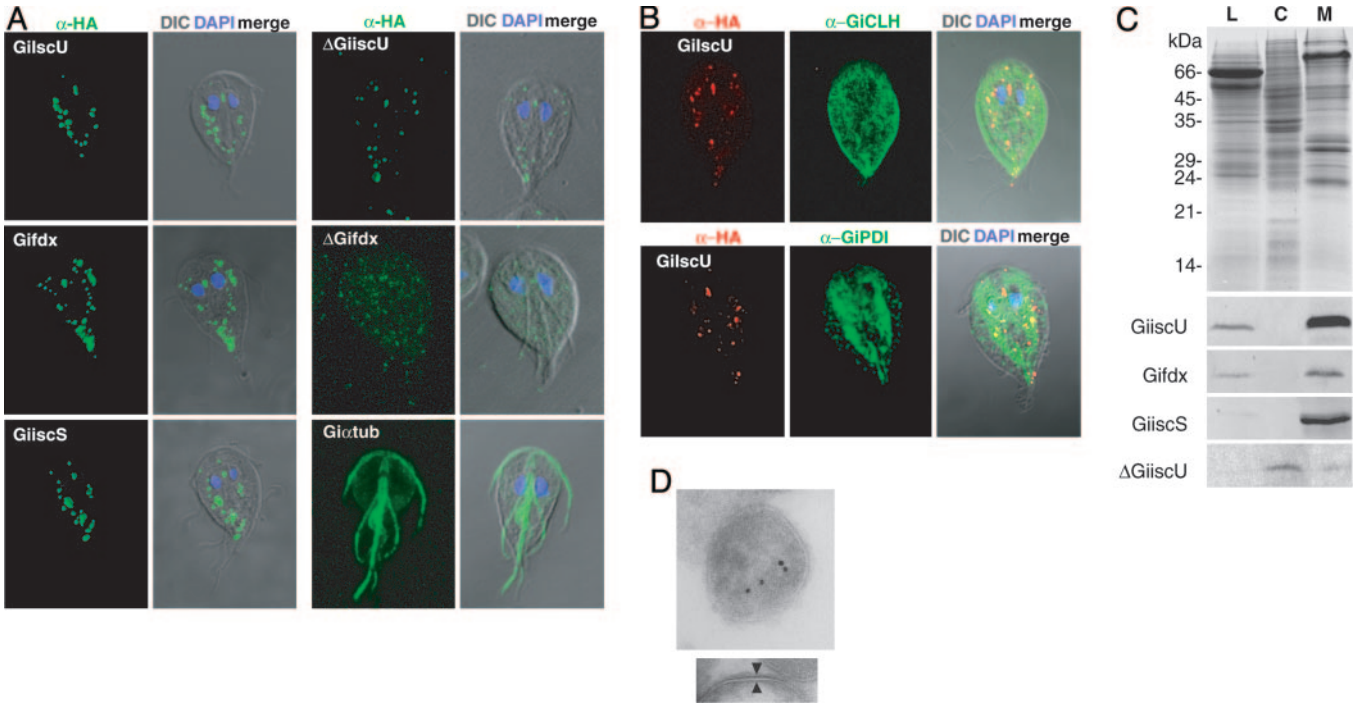

Fig. 2. Cellular localization of tagged GiiscU, GiiscS, and Gifdx in *G. intestinalis* transformants. (*A*) Transformed *Giardia* cell lines were stained for immunofluorescence microscopy with mouse α-HA tag Ab (green). GiiscU and Gifdx, the complete preproteins possessing N-terminal presequences; ΔGiiscU and ΔGifdx, truncated forms lacking the N-terminal presequences. GiiscS does not possess recognizable N-terminal presequence. *Giardia* α-tubulin (Giαtub) was used as a control. Merged images are given for immunofluorescent staining, the nuclei (blue) stained with DAPI, and differential interference contrast (DIC). (*B*) Mitosomes stained for GiiscU (red); peripheral vesicles and structures of endoplasmic reticulum stained for clathrin light chain (α-GiCLH Ab) and protein disulfide isomerase (α-GiPDI Ab), respectively, (green). (*C*) Total cell lysate (L), cytosolic (C), and mitosomal (M) fractions were prepared from transformed cells and analyzed by SDS/PAGE (*Top*) and Western blots (bottom four blots). (*D*) Immunoelectron microscopy of the mitosomes purified from *giiscU* transformants. Tagged GiiscU was detected in the organelles by the mouse α-HA Ab and 10 nm gold-labeled goat α-mouse Ab. Arrowheads indicate the double (outer and inner) membranes of the mitosome.

examine which part of the 434-aa protein is required for targeting to mitosomes, the protein was truncated and the N-terminal 202 residues (GiiscSN1/2) or C-terminal 232 residues (GiiscSC1/2) expressed in *T. vaginalis*. Both fragments of the protein were delivered into the hydrogenosomes (Fig. 3*C*). These results indicate that IscS contains multiple targeting signals within the protein.

Conservation of Protein Targeting in Mitosomes and Hydrogenosomes. To determine whether the mitosomal targeting sequences on GiiscU and Gifdx can function to target proteins to hydrogenosomes, the giardial genes were overexpressed in *T. vaginalis.* Immunofluorescence labeling of trichomonad cells expressing tagged GiiscU, Gifdx, and GiiscS localized these proteins to discrete structures surrounding trichomonad nuclei and cytoskeletal structures, the cell distribution typical for hydrogenosomes (Fig. 3*A*). The labeling of tagged proteins also colocalized with malic enzyme, a marker protein for hydrogenosomes. Stronger malic enzyme signal corresponds to its abundance in hydrogenosomes (30). In contrast, the absence of N-terminal leader sequences on ΔGiiscU and ΔGifdx abrogated the delivery of the proteins into the target organelle with the majority of each protein accumulating in the cytosol (Fig. 3*B*).

The N-terminal extension of GiiscU is not only necessary, but sufficient, for targeting of this protein into the hydrogenosomes. Attaching the extension of GiiscU to the N terminus of α-tubulin delivers a significant proportion of this passenger protein into the hydrogenosomes, whereas no giardial α-tubulin was found in the organelles when expressed without the GiiscU targeting sequence (Fig. 3*B*).

Processing of a Mitosomal Targeting Sequence. The detection of GiiscU expressed in *T. vaginalis* hydrogenosomes revealed the presence of two bands of 20 and 17 kDa corresponding to the predicted molecular mass of the GiiscU precursor and its mature form, respectively. In *Giardia*, the size of tagged GiiscU detected in mitosomes of the cells expressing the complete *giiscU* was identical to its truncated form expressed in Δ*giiscU* transformants. These observations indicated processing of N-terminal targeting sequence within the target organelles (Fig. 4*A*). To test whether specific metallopeptidases, which are known to mediate cleavage of targeting sequences in mitochondria (33) and possibly in hydrogenosomes (23), can process the giardial targeting sequences, we incubated *in vitro* translated GiiscU preprotein with lysates of yeast mitochondria or trichomonad hydrogenosomes. The mitochondrial lysate efficiently catalyzed the cleavage of GiiscU in a time-dependent manner (Fig. 4*B*), as did the hydrogenosomal extract (Fig. 4*C*). The cleavage was inhibited by the addition of EDTA, indicating that a metalloprotease is involved. Pretreatment of the hydrogenosomal lysate with hexokinase to remove ATP did not affect the cleavage, which excludes a possibility that the observed processing was catalyzed by ATP-dependent proteases. To determine the protein cleavage site, GiiscU preprotein was incubated with recombinant rat MPP (Fig. 4*D*). The N-terminal sequence of the major cleavage product (inhibitable by EDTA) revealed that the MPP cleaved the GiiscU precursor between Phe-18 and Leu-19 with arginine at −2 position (Fig. 1). Finally, overexpressed GiiscU was immunoprecipitated from a giardial high-speed pellet to verify whether native cleavage site in *Giardia* corresponds to that catalyzed by recombinant MPP (supporting information). Indeed, the N-terminal sequence of the GiiscU retrieved from

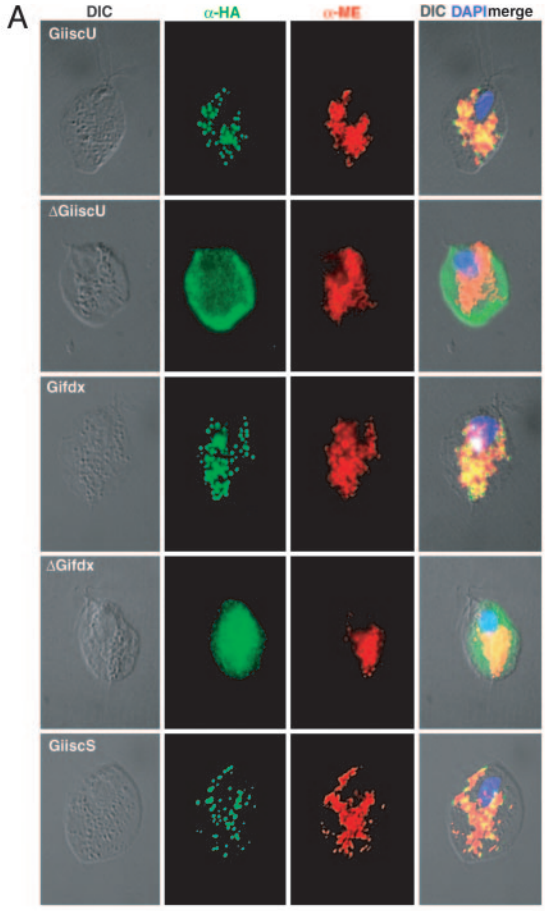

Fig. 3. Targeting of the mitosomal proteins GiiscU, Gifdx, and GiisS into *T. vaginalis* hydrogenosomes. (*A*) The *Giardia* proteins were each expressed in trichomonads with a C-terminal HA tag (green). A rabbit polyclonal Ab recognizing the hydrogenosomal malic enzyme (α-ME) was used as a marker for hydrogenosomes (red). The nuclei were stained with DAPI (blue). DIC, differential interference contrast. (*B*) Total cell lysate (L), cytosol (C), and hydrogenosomal (H) fractions were prepared from transformed *Trichomonas* cells and analyzed by SDS/PAGE and Western blots.

Giardia started with Leu-19 (Fig. 1). Although it is not yet clear how generally applicable PSORT (http://psort.nibb.ac.jp) will be for predicting cleavage sites in mitosomal proteins, in the case of GiiscU, the prediction was successful.

Homologs of the β-subunit of MPP are encoded in the genomes of *G. intestinalis* and *T. vaginalis*. Both of these β-MPP-like sequences possess characteristic His-X-X-Glu-His zinc-binding motifs and PSORT-predicted presequences (supporting information). Overexpression of β-MPP in *G. intestinalis* showed its colocalization with GiiscU in mitosomes (Fig. 5).

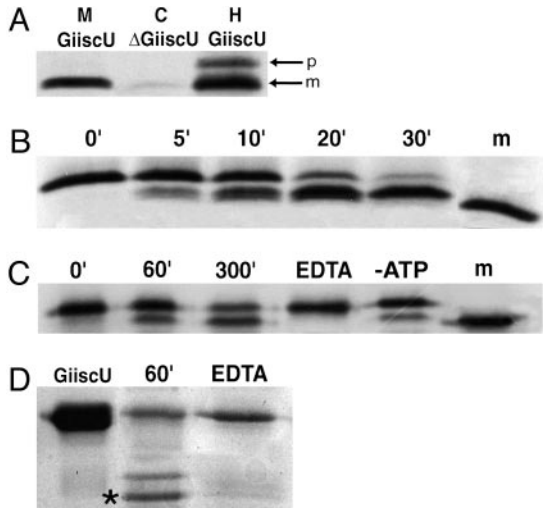

Fig. 4. Processing of GiiscU. (*A*) M, GiiscU detected in mitosomes of *Giardia*; C, N-terminal truncated form of GiiscU retained in the cytosol of Δ*giiscu* transformants; H, two forms of GiiscU in the hydrogenosomes represent the precursor (p) and the processed (m) protein. (*B*) Time-dependent processing of *in vitro* translated, ^{35}S-labeled GiiscU precursor in the presence of yeast mitochondrial lysate. The mature-sized form of GiiscU (m). (*C*) GiiscU precursor was ^{35}S-labeled and incubated with lysate prepared from *T. vaginalis* hydrogenosomes with or without 10 mM EDTA. ATP-depleted lysate (−ATP) by using hexokinase (34). (*D*) GiiscU precursor incubated for 60 min with rat MPP (33). The N terminus of major cleavage product (asterisk) was determined by microsequencing.

A Subunit of the Mitochondrial PAM Complex in Mitosomes and Hydrogenosomes. Although only partial information is available on the genome sequence from *G. intestinalis* and *T. vaginalis*, we have initiated iterative BLAST analyses (29) to search for homologs of the components of the mitochondrial protein import machinery. The Pam18-related sequences in Fig. 5*C* all predict to have a transmembrane segment, followed by a conserved charged region and a J domain characterized by the absence of a predicted "helix IV" (37). To determine their subcellular location, GiPam18 and TvPam18 were expressed as HA-tagged proteins in *G. intestinalis* and *T. vaginalis*. The proteins of both origins were delivered into both hydrogenosomes and mitosomes and colocalized with the marker proteins, GiiscU and malic enzyme, respectively (Fig. 5 *A* and *B*).

Discussion

Our study provides evidence that mitosomes of *G. intestinalis*, hydrogenosomes, and mitochondria share a similar mode of protein targeting and translocation. This finding supports the hypothesis that mitosomes, hydrogenosomes, and mitochondria represent different forms of the same fundamental organelle that have evolved under distinct selection pressures (38).

Three giardial proteins were selected to compare their targeting and translocation into mitosomes and hydrogenosomes: GiiscS, GiiscU, and [2Fe2S] ferredoxin (Gifdx). These proteins are homologues of the key components of the FeS cluster assembly machinery operating in mitochondria and hydrogenosomes (11, 12). Association of GiiscS and GiiscU with giardial mitosomes has been reported in ref. 4, whereas the intracellular localization of Gifdx has not been studied. When GiiscS, GiiscU, and Gifdx were overexpressed in *G. intestinalis* or *T. vaginalis*, all three proteins were specifically delivered into the mitosomes or into the hydrogenosomes, respectively. The delivery of the proteins was mediated by two different mechanisms requiring either N-terminal targeting sequences (Gifdx, GiiscU) or internal targeting sequences (GiiscS). The N-terminal extensions

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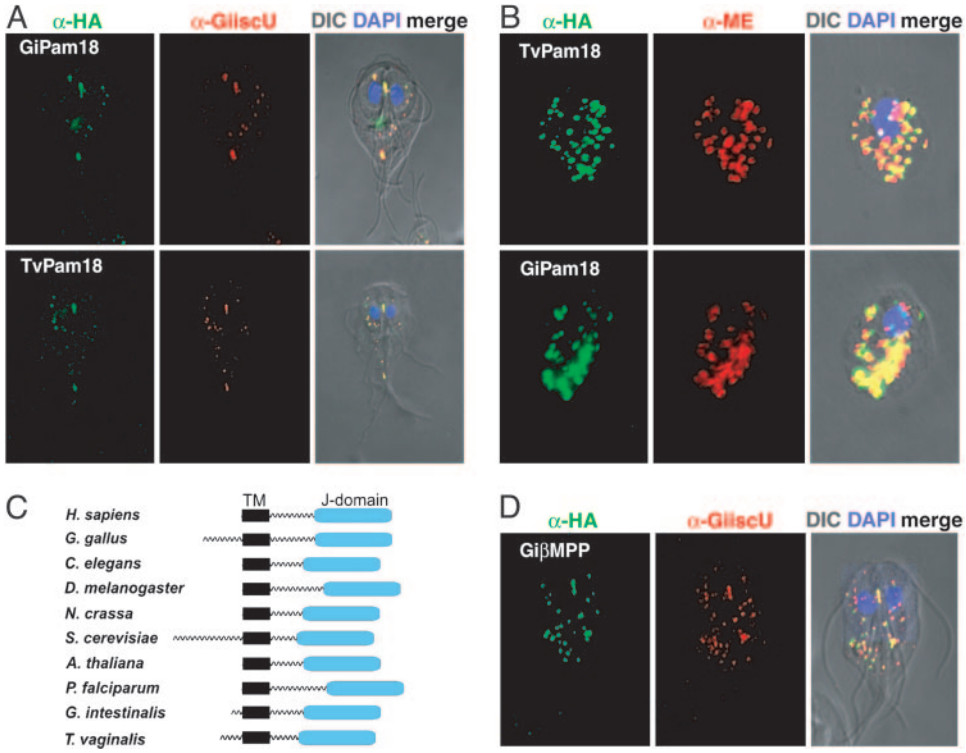

Fig. 5. Cellular localization of tagged GiPam18, TvPam18, and GiβMPP. Transformed cell lines of *G. intestinalis* (*A* and *D*) and *T. vaginalis* (*B*) were stained for immunofluorescence microscopy with mouse α-Ha Ab (green). GiiscU was detected by polyclonal rabbit α-GiiscU Ab (red). The merged images are given for immunofluorescent staining, the nuclei (blue) stained with DAPI. (*C*) Domain structure of GiPam18 and TvPam18 compared with other members of the Pam18/Tim14 family. Ten of the most diverse sequences were aligned with CLUSTALW (supporting information). The N-terminal extension segments were located in the intermembrane space (20), transmembrane segments (TM) were predicted with DAS (36), and the J domain (blue) was characterized as described in ref. 37.

predicted in GiiscU and Gifdx are both necessary and sufficient for targeting to mitosomes and resemble the targeting sequences found in mitochondrial and hydrogenosomal proteins in that they (*i*) are rich in serine and arginine residues, (*ii*) are predicted to form amphipathic helices, and (*iii*) possess cleavage site motifs recognized by mitochondrial-type processing peptidases.

In mitochondria, MPP is an EDTA-sensitive metalloprotease that consists of two subunits. Genes coding for α and β subunits of MPP can be found widely in eukaryotes, including animals, fungi, and plants (22). Although the hydrogenosomal processing peptidase has not been biochemically characterized, protein processing in hydrogenosomes was observed (23), and sequences for each subunit of MPP are annotated in the *T. vaginalis* genome. We found a putative β-MPP subunit in the *Giardia* genome and showed that the protein is delivered into mitosomes. EDTA-sensitive cleavage of the GiiscU N-terminal targeting sequence was observed with purified rat MPP and with hydrogenosomal extracts, and analysis of GiiscU isolated from *Giardia* indicated that protein processing also occurred *in situ*.

Protein targeting sequences and their processing peptidase are common in mitosomes, hydrogenosome and mitochondria. Are the fundamental components of the TOM and TIM complexes also to be found in *Trichomonas* and *Giardia*? It is clear now that although some components of the mitochondrial protein import machinery might have evolved after the radiation of the main eukaryotic lineages (39), several components of the TOM (29) and TIM (40) complexes were likely present at the earliest stage in the conversion of the endosymbiont that gave rise to mitochondria. Our data predicts that these primitive components of the TOM and TIM complexes will be present in hydrogenosomes and mitosomes. In at least one case, Tim14/Pam18, this prediction has been fulfilled. Although there are 26 different proteins containing J domains in yeast, only Pam18/Tim14 (and its paralog Mdj2) contain a transmembrane segment, a charged linker domain, and a J domain without the characteristic "helix IV" (37). The function of Tim14/Pam18 is to dock to the TIM23 complex, assist Tim44 to bind the mitochondrial Hsp70, and to directly stimulate ATP hydrolysis catalysed by Hsp70 to promote protein translocation across the mitochondrial membranes (20, 21). We do not currently have an assay system capable of dissecting the function of the Pam18-related proteins of *Giardia* and *Trichomonas*, but the presence of GiPam18 in mitosomes and TvPam18 in hydrogenosomes provides an indication that the protein translocation machinery of these organelles and mitochondria might be built around commonly derived components. More sensitive means of sequence analysis may be required to identify further subunits of the TOM and TIM complexes, and we have initiated studies to build hidden Markov models for this purpose.

The endosymbiotic event of an α-proteobacterium that gave rise to mitochondria and related organelles is of great interest because this event might represent the moment of the origin of the eukaryotic cell itself (41). Studying the fate of the ancestral endosymbiont in different eukaryotes promises to uncover the nature and primary role of the organelle for eukaryotes. The fact that hydrogenosomes and mitochondria recognize the targeting signals of mitosomal proteins indicates that these organelles possess a common protein import mechanism and suggests that all these organelles share, through common descent, what must have been among the earliest features of the first "mitochondriate" organisms.

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