Mitochondrial remnant organelles (mitosomes) that exist in a range of “amitochondrial” eukaryotic organisms represent ideal models for the study of mitochondrial evolution and for the establishment of the minimal set of proteins required for the biogenesis of an endosymbiosis-derived organelle. *Giardia intestinalis*, often described as the earliest branching eukaryote, contains double membrane-bounded structures involved in iron-sulfur cluster biosynthesis, an essential function of mitochondria. Here we present evidence that *Giardia* mitosomes also harbor Cpn60, mtHsp70, and ferredoxin and that despite their advanced state of reductive evolution they have retained vestiges of presequence-dependent and -independent protein import pathways akin to those that operate in mammalian mitochondria. Although import of IscU and ferredoxin is still reliant on their amino-terminal presequences, targeting of *Giardia* Cpn60, IscS, or mtHsp70 into mitosomes no longer requires cleavable presequences, a derived feature from their mitochondrial homologues. In addition, we found that division and segregation of a single centrally positioned mitosome tightly associated with the microtubular cytoskeleton is coordinated with the cell cycle, whereas peripherally located mitosomes are inherited into daughter cells stochastically.

Highly derived but functional mitochondrial remnant organelles (mitosomes) are found in “amitochondrial” eukaryotes as diverse as *Entamoeba histolytica*, *Trachipleistophora hominis*, *Cryptosporidium parvum*, *Blastocystis hominis*, and *Giardia intestinalis* (1–5). Although able to metabolize small amounts of oxygen, these organisms live mostly in oxygen-deprived environments and have lost their capacity for ATP biosynthesis linked to oxidative phosphorylation, one of the key mitochondrial marker proteins Cpn60, mtHsp70, ferredoxin (Fd), IscS, and IscU reside in *Giardia* mitosomes. In *vivo* organelle targeting experiments showed that only IscU and Fd require the presence of amino-terminal targeting presequences, which are cleavable and functionally conserved with those of mammalian mitochondria. Finally, we found that a single central mitosome actively divides and segregates during mitosis, whereas peripheral organelles are not coordinately replicated and segregate into daughter cells stochastically.

**EXPERIMENTAL PROCEDURES**

*Parasite Cultivation and Transfection—*Trophozoites of the *G. lamblia* strain WB were grown in TYI-S-33 medium supplemented with 10% adult bovine serum and bovine bile as described previously (3, 59). Incubations with nocardazole or cytochalasin D (10 μg/ml) added as 100× stock solutions in Me2SO to normal growth medium were done for up to 2 h at 37 °C. Parasites were harvested by chilling culture tubes on ice for 30 min to detach adhering cells and centrifugation at 800 × g. Transgenic *Giardia* lines were generated by electroporation in the presence of indicated plasmid DNA and appropriate drug selection (60).

*Construction of Expression Vectors—*Standard recombinant DNA techniques were used for nucleic acid preparation and analysis. For full details on the construction of conditional and constitutive recombinant

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*This work was supported in part by Swiss National Science Foundation Grant 3100A0-100270 (to A. B. H.) and by Wellcome Trust Grant 059845 (to J. T.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The on-line version of this article (available at http://www.jbc.org) contains four supplemental tables.

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Expression vectors used in this study see supplemental data. 

Cultivation and Transfection of Mammalian Cells—Human embryonic kidney 293 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum at 37 °C in a humidified 5% CO₂ atmosphere. Cells of 20–30% confluence in 6-well plates were transfected with plasmid DNA using FuGENE 6 transfection reagent (Roche Applied Science) according to the manufacturer’s protocol. Transfected cells were incubated for 18 h and subsequently treated with 0.25% trypsin in TDE buffer (136 mM NaCl, 50 mM KCl, 50 mM EDTA, 25 mM Tris, pH 7.5) for 2 min, detached by resuspension, centrifuged at 500 × g for 10 min, and resuspended in 1.2 ml of fresh Dulbecco’s modified Eagle’s complete medium. Cells (8.0 × 10⁴) were applied on each glass slide and attached to the surface for 5 h at 37 °C in a 5% CO₂ incubator. Following removal of medium and addition of 500 μl of fresh medium containing 125 nm MitoTracker Red (Molecular Probes), slides were incubated at 37 °C for 15 min, washed twice in PBS, fixed in 4% paraformaldehyde for 30 min at 37 °C, and processed for microscopy as described below.

Laser Scanning Confocal Immunofluorescence Microscopy—After treatments, human embryonic kidney 293 cell-containing slides were washed five times in PBS and mounted with VectaShield mounting medium. Slides were observed under a scanning confocal microscope (Radiance 2100; Bio-Rad). Images were collected using Lasersharp software and further processed with Adobe Photoshop 7.0. In the case of parasites, chilled cells were harvested as described above, washed with ice-cold PBS, and fixed with 3% formaldehyde for 40 min at room temperature, followed by a 5-min incubation with 0.1 M glycine in PBS. Fixed cells were permeabilized with 0.2% Triton X-100 in PBS for 20 min and blocked >2 h in 2% bovine serum albumin in PBS. Incubations with titrated primary or secondary antibodies were performed in 2% bovine serum albumin/0.2% Triton X-100 in PBS. Cells were washed with PBS between incubations and embedded with Vectashield containing DAPI (Vector Labs). Image stacks were collected on a Leica SP2 AOBS confocal laser scanning microscope (Leica Microsystems, Wetlar, Germany) using the appropriate settings. Color merging was done using the Leica software or the Metaview software package (Visitron Systems, Puchheim Germany). In some cases images were further processed using the Imaris software (Bitplane) in conjunction with the Huygens package for deconvolution.

Production of Polyclonal Antisera in Mice—Polyclonal antisera against GiCpn60 were produced as described previously (20). For details see supplemental data.

RESULTS

Localization of Cpn60 and mtHsp70 Corroborates the Endosymbiotic Origin of the Relict Organelles of Giardia—The cellular localization of mitochondrial marker proteins such as Cpn60 and mtHsp70 has not been demonstrated unequivocally in Giardia. The apparent extra-mitosomal distribution of Cpn60, as determined by confocal microscopy with heterologous anti-Cpn60 antibodies, is highly controversial (3, 18, 19). We have used specific homologous antibodies raised against a bacterially expressed GiCpn60 fusion protein and ectopic expression of an epitope-tagged variant of Cpn60 (Cpn60-HA) to establish the cellular distribution of this protein in parasite trophozoites by confocal immunofluorescence microscopy (IFA). Mouse sera that reacted specifically with a single band of ~60 kDa. Relative masses of marker proteins are indicated in kilodaltons. B–G, subcellular localization of endogenous and recombinant mitosome matrix proteins by immunofluorescence. Nuclear DNA is stained with DAPI and appears blue. All micrographs were produced with a confocal laser scanning microscope. Scale bars are 2 μm. B, giardial Cpn60 (red) detected by the anti-Cpn60 antibody shows the typical distribution of mitosome proteins, including labeling of the elongated organelle associated with the basal body complex (arrowhead). C, co-localization of GiCpn60 (green) and the mitosome marker Ics5 (red). D and E, ectopically expressed Cpn60-HA or GimpHSP70 co-localize with Ics5 in mitosomes. F and G, mitosomes do not overlap with the two other major membrane compartments, the peripheral vesicles and the endoplasmic reticulum. Peripheral vesicles are defined by peripherally associated clathrin; the endoplasmic reticulum is labeled with antibodies against a giardial protein disulfide isomerase (2).

FIG. 1. Giardia Cpn60 is a mitosomal protein. A, Western blot of Giardia Triton X-100 soluble proteins separated by SDS-PAGE and probed with a polyclonal antibody against recombinant GiCpn60 revealed a single band of ~60 kDa. Relative masses of marker proteins are indicated in kilodaltons. B–G, subcellular localization of endogenous and recombinant mitosome matrix proteins by immunofluorescence. Nuclear DNA is stained with DAPI and appears blue. All micrographs were produced with a confocal laser scanning microscope. Scale bars are 2 μm. B, giardial Cpn60 (red) detected by the anti-Cpn60 antibody shows the typical distribution of mitosome proteins, including labeling of the elongated organelle associated with the basal body complex (arrowhead). C, co-localization of GiCpn60 (green) and the mitosome marker Ics5 (red). D and E, ectopically expressed Cpn60-HA or GimpHSP70 co-localize with Ics5 in mitosomes. F and G, mitosomes do not overlap with the two other major membrane compartments, the peripheral vesicles and the endoplasmic reticulum. Peripheral vesicles are defined by peripherally associated clathrin; the endoplasmic reticulum is labeled with antibodies against a giardial protein disulfide isomerase (2).

Co-localization studies using antibodies against established markers for peripheral vesicles (clathrin, GiCLH) (Fig. 1F) and endoplasmic reticulum (protein disulfide isomerase 2, GiPD2) (Fig. 1G). Analysis by confocal microscopy confirmed that mitosomes are independent organelles not related to the endoplasmic reticulum or the endosomal-lysosomal membrane system (20). These data, together with the phylogeny of the giardial Cpn60, mtHsp70, and Ics5 (16, 21–24), strongly support the mitochondrial ancestry of Giardia mitosomes.

Conserved and Diverged Features of Protein Import Pathways in Giardia Mitosomes—In contrast to their homologues from other eukaryotes, giardial Cpn60, mtHsp70, and Ics5 lack
putative amino-terminal targeting presequences (16, 21–23). Their mitosomal localization, however, suggests that these proteins are imported into the organelle via non-cleavable targeting signals that may be equivalent to those from a number of luminal mitochondrial proteins such as oxacyl-CoA thiolase and chaperonin 10 (25–27). In contrast, putative organelle targeting presequences have been identified in two Giardia proteins, IscU and Fd, but their functionality has not been demonstrated (3, 28). We tested the requirement for these putative targeting presequences using tagged variants of IscU and Fd fused either to HA or to green fluorescent protein (GFP). Removal of amino acids 2–27 (Thr2-Glu27) from GiIscU-HA led to the accumulation of truncated protein in the cytoplasm and was completely excluded from mitosomes (Fig. 2, A and B). Similarly, deletion of amino acids 2–26 (Thr2-Asp26) from GiIscU-GFP and of amino acids 2–18 (Ser2-Gln18) from GiFd-GFP led to accumulation of protein in the cytosolic fraction (Fig. 3A). Deletion of amino acids 2–5 (Leu2-Thr5) from the amino terminus of Cpn60-HA, which contains no predicted targeting presequence and is only 2 amino acids longer than that of its bacterial homologues (16), had no effect on its mitosomal localization (Fig. 2C). Together, these data demonstrate that presequence-dependent and -independent protein import pathways still operate in Giardia mitosomes, despite their advanced state of reductive evolution.

Processing of IscU and Fd Amino-terminal Presequences—A functional feature of mitochondrial and hydrogenosomal protein import is the proteolytic removal of targeting peptides upon organelle import (29, 30). To investigate whether the amino-terminal peptides of Giardia IscU and Fd are cleaved upon import into mitosomes, the initial 26 and 18 amino acids of IscU (Met1-Asp26) and of Fd (Met1-Gln18), respectively, were fused to the passenger protein GFP. Transfection of these constructs into Giardia trophozoites resulted in very high levels of GFP expression, which appeared evenly distributed throughout the cell in confocal images (not shown). However, cell fractionation experiments clearly demonstrated the accumulation of the full-length 5′-IscU-GFP and 5′-Fd-GFP fusion proteins in the cytosolic fraction, whereas mature GFP, which co-migrated with GFP from control parasite lines, was found exclusively in the organelar fraction (Fig. 3B). Such distribution of precursor and processed proteins strongly suggests that the amino-terminal targeting signals of Giardia IscU and Fd are removed upon organelle import and that in vivo protein import into mitosomes is an active saturable process. Western blot analysis of parasite subcellular fractions using specific anti-IscU antibodies confirmed that the native Giardia protein is indeed processed upon mitosome import (Fig. 3C) as indicated by the presence of a major band of ~14.5 kDa (mature protein) and a...
minor band of ~17 kDa (precursor protein) consistent with the removal of the targeting peptide from the precursor protein. Together, these data demonstrate that mitosome protein import, like protein import into mitochondria and hydrogenosomes, is a saturable process that requires, at least in part, the presence of cleavable amino-terminal presequences.

**Functional Conservation of Targeting Signals in Giardia Mitosomes and Mammalian Mitochondria**—The requirement of cleavable targeting presequences for mitosomal protein import suggests that the mechanisms of giardial mitosome protein import could be functionally conserved with those of mitochondria. To test this hypothesis we cloned the 5′-Fd-GFP fusion construct into a mammalian expression vector and transiently transfected human embryonic kidney cells. The passenger GFP protein was efficiently targeted into mammalian mitochondria by the Giardia Fd presquence, as demonstrated by the co-localization of recombinant GFP with the mitochondrial marker dye MitoTracker Red (Fig. 4, upper panels). In the absence of the mitosomal targeting presquence, GFP accumulated in the cytosol (lower panels). These data demonstrate the functional conservation of targeting presequences between Giardia mitosomes and mammalian mitochondria and suggest the existence of common protein import pathways in these organelles.

**Organelle Replication and Inheritance**—In confocal microscopy images Giardia mitosomes appear as ~100-nm spherical compartments randomly distributed throughout the cytoplasm with concomitant staining of a distinct rod-like structure invariably localized in the medial axis between the nuclei. Based on their cellular distribution we have dubbed these structures peripheral mitosomes (PMs) and central mitosomes (CMs), respectively. To investigate how PMs and CMs replicate and segregate, fluorescence microscopy was used to analyze their distribution at different stages of the cell cycle. The single CM localized exclusively to the basal body region between the nuclei during interphase (Fig. 5A). In mitotic cells, however, doubling and segregation of CMs was consistently observed as an early event prior to the onset of karyokinesis (Fig. 5, B and D), suggesting that replication and inheritance of CMs is coordinated in a cell cycle-dependent manner. To assess the behavior of PMs during the cell cycle, photomicrographs of interphase and of mitotic cells were used to quantify the number of PMs present in each cell. Statistical analysis showed that the mean number of PMs was slightly higher in mitotic cells than in interphase trophozoites (mean values 57.6 and 50.9/cell, respectively, n = 40) (Fig. 5C). Although significant (p < 0.05), the difference is far too small to account for the number of organelles that would be expected in mitotic cells if replication of PMs were coordinated with the cell cycle. Rather, our data suggest that PMs are not coordinately replicated and that they segregate during cell division stochastically.

The close association of CMs with the basal body region suggested the involvement of the cytoskeleton in organelle positioning, division, and inheritance. To investigate the contribution of the cytoskeleton to these processes, trophozoites were treated for 2 h with cytochalasin D or with nocodazole, specific inhibitors of actin filaments and microtubule assembly, respectively. Cytochalasin D did not change the appearance and localization of CMs, although PMs appeared to be more abundant in these cells (Fig. 5B). Because actin filaments have never been detected in Giardia, the reason for this effect remains unexplained. Conversely, addition of nocodazole to the culture medium resulted in de-localization of the CMs, whereas the distribution of PMs appeared unaffected (Fig. 5B). These data demonstrate a direct physical connection between CMs and the microtubular cytoskeleton.

We established a sequence of events for CM duplication and segregation during mitosis using Cpn60 as a marker of mitosomes (Fig. 5D). In agreement with a recent investigation (31), we found that cell division follows a ventral-dorsal pattern with preservation of orientation and handedness in the daughter cells. The cell in the left panel (Fig. 5D) represents the earliest detectable stage of mitosis (stage I). Typical features were the beginning assembly of a daughter ventral disk and elongated nuclei with the DNA appearing condensed into 8–10 limbs. The CM was duplicated very early, and the two organelles were separated but still in close proximity. During stage II sequential karyokinesis of both nuclei was observed (32), and both the basal bodies of the axonemes and CMs became clearly segregated into daughter cells. In stage III cytokinesis was completed and daughter cells received one CM and a set of PMs each. Such close association between CMs and the cytoskeleton during the cell cycle suggests similarities with mitochondrial replication and inheritance in Trypanosoma brucei, as discussed below.

**DISCUSSION**

Until the recent discovery of mitosomes, Giardia had been believed to be devoid of organelles of endosymbiotic origin, either through secondary loss or because branching of the diplomonad ancestor had occurred before the establishment of mitochondria in the eukaryotic lineage. Contrary to the proposed mitochondrial ancestry of mitosomes, however, electron and confocal microscopy images obtained using mammalian- and Entamoeba-specific anti-Cpn60 antibodies suggested that this key marker of mitochondria might not co-localize with Isc proteins in Giardia mitosomes or even be associated with biological membranes (3, 18). Data presented in this report settle the controversy (19) by demonstrating unequivocally that all these mitochondrial markers do indeed reside in mitosomes. Our findings substantiate the hypothesis that these organelles represent vestigial mitochondria and demonstrate the impor-
tance of using homologous antibodies when working with highly derived organisms such as *Giardia*.

The biosynthesis and assembly of iron-sulfur clusters is carried out in the lumen of mitochondria, hydrogenosomes, and *Giardia* mitosomes. Most of the proteins that reside and function in the lumen of mitochondria and hydrogenosomes are imported into their respective organelles via positively charged and cleavable amino-terminal targeting presequences (29, 30). Targeting of GiIscU and GiFd into *Giardia* mitosomes was also found to be dependent on their amino-terminal presequences, which are cleaved off from precursor proteins upon import into mitosomes. Furthermore, mitosomal targeting presequences are readily recognized by the protein import machinery of mitochondria, thus demonstrating a conservation of function between protein import pathways in both organelles. Despite their highly degenerate nature and despite the difficulty in identifying homologues of the protein translocases of mitochondria in *Giardia* mitosomes (see below), these organelles have clearly retained the molecular components required to translocate proteins from the cytosol in a presequence-dependent manner. Evidence for the functional conservation of mitochondrial and mitosomal targeting signals has also been obtained in *Entamoeba* and *Cryptosporidium* (2, 33–38). Such apparent widespread conservation of function in mitosomal and mitochondrial protein targeting, together with the mutually exclusive distribution of mitosomes and mitochondria in eukaryotic lineages, strongly supports the mitochondrial ancestry of mitosomes.

Mitochondrial and hydrogenosomal IscS, mtHsp70, and Cpn60 all require cleavable amino-terminal targeting presequences for import into their respective organelles (39–43). In *Giardia*, however, we found that this feature is no longer conserved as all three proteins are efficiently targeted into mitosomes, alone or as fusion proteins, in the absence of amino-

![Subcellular localization and inheritance of the central mitosome during the cell cycle.](image-url)

**Fig. 5.** Subcellular localization and inheritance of the central mitosome during the cell cycle. A, close-up differential interference contrast (DIC) and fluorescence images of a CM stained with anti-GiCpn60 showing association with the basal body complex of *Giardia*. CF, caudal flagella; VF, ventral flagella; BB, basal bodies of the six posterior flagella; N, nuclei; CM, central mitosome; PM, peripheral mitosomes. B, distribution and subcellular localization of all PMs and the CMs in a projection image of optical sections. Left panel, interphase cell. Second panel, postmitotic cell with divided CM. Third panel, treatment with the actin-depolymerizing agent cytochalasin D does not affect the subcellular localization of the CM. Right panel, the CM become delocalized in cells treated with the microtubule-depolymerizing drug nocodazole, but PMs appear unchanged. The outlines of the nuclei are shown in red. Blue arrowheads, CM localization. C, statistical analysis of PM numbers in dividing cells. Gray bars, mean number of PMs in dividing and interphase cells (n = 40); line bars, standard deviation. D, representative images showing CM localization (yellow arrowheads) during three stages of mitosis and cell division (I-III). Micrographs show three-dimensional reconstructed confocal images (25 optical sections/stack). Nuclei are stained with DAPI and are depicted in green for enhanced contrast. DIC images, layer 12/25. Scale bars are 2 μm.
terminal targeting presequences (16, 21–23). Further, removal of the 5 initial amino acids of GiCpn60 did not preclude its import into mitosomes, demonstrating that Giardia Cpn60 is not constrained by the amino-terminal peptide requirement displayed by its mitochondrial and hydrogenosomal homologues. Together with the identification of only two proteins with cleavable amino-terminal targeting presequences in Giardia (IscU and Fd), these data illustrate the extent of reductive evolution already imposed on the presequence-dependent protein import pathway of Giardia mitosomes. Import of a mHTsp70 protein without an obvious organelle targeting presequence has also been observed in T. hominis, another mitosome-containing organism (1). Such a derived feature resembles the presequence-independent import mechanism observed in a number of luminal mitochondrial proteins such as rhodanese, 3-oxoacyl-CoA thiolase, and chaperonin 10 (27). This might represent an ancestral feature of organelle protein import as some bacterial proteins appear to have a natural predisposition for organelle targeting (44).

The protein import machinery of mitochondria is known to contain dozens of proteins, including cytosolic chaperones, membrane-bound protein translocases, and accessory luminal mitochondrial proteins such as molecular chaperonins and processing peptidases (29). The number of cellular proteins required for hydrogenosomal protein import is as yet unknown, processing peptidases (29). The number of cellular proteins such as molecular chaperonins and membrane-bound protein translocases, and accessory luminal components were tentatively identified, from protozoa to man, and including plant sequences (supplemental information). The protein import machinery of mitochondria is known to contain dozens of proteins, including cytosolic chaperones, membrane-bound protein translocases, and accessory luminal mitochondrial proteins such as molecular chaperonins and processing peptidases (29). The number of cellular proteins required for hydrogenosomal protein import is as yet unknown, but this process shares many of the physiological requirements of mitochondrial protein import (30, 45, 46). It is now evident that Giardia must also possess a considerable number of proteins that participate in mitosome protein import and organelle division, yet the number of putative mitosomal proteins glanced from the fully sequenced Giardia genome remains pitifully low (47). Fig. 6 is a working model and provides a graphical representation of a data base search for components of the protein translocation machinery (for experimental details see supplemental data). Each of the subunits detailed in Fig. 6 was searched in the Giardia Genome Database using query sequences from a wide variety of organisms, ranging from protozoa to man, and including plant sequences (supplemental information). Tables S2-S4). Although two candidate components were tentatively identified, i.e. the GiTOM70 subunit (GenBank™ EAA41006) and a mitochondrial protein peptidase homologue (GenBank™ EAA39560), the genomic data alone were insufficient to reconstruct protein import machinery derived from a mitochondrial endosymbiont. The use of more refined bioinformatics tools and of alternative biochemical and biophysical protein identification methods, including organelle proteomics and mass spectrometry, will be required for the identification of the molecular components that participate in mitosome protein import and organelle replication.

A unique feature of Giardia mitosomes is their distinct morphological manifestations within a cell, i.e. a unique centrally located rod-shaped structure and the more abundant spherical organelles distributed throughout the cytoplasm. Interestingly, only the CM is actively inherited in a cell cycle-dependent manner, while the peripheral organelles appear to be maintained at relatively constant numbers and segregate into daughter cells stochastically. Exactly how PMs of Giardia are replicated and maintained in constant numbers in the cell is at present unclear. One possibility is that PMs might arise from the ends of CMs in a process analogous to the fission events observed in yeast and mammalian mitochondria where, through the participation of dynamin-related mechanoenzymes, complex mitochondrial networks can give rise to individual organelles (48–50). A second possibility is that individual PMs may divide uncoordinatedly by binary fission or septation, as observed in trichomonad hydrogenosomes (51). Further research is required to unequivocally differentiate between these possibilities.

CMs are tightly associated with the basal bodies of flagellar axonomes, which are known to replicate early during mitosis (52), and depend on the integrity of the microtubular cytoskeleton for positioning and replication as demonstrated by their dramatic de-localization in nocodazole-treated trophozoites. These features of mitosome replication and inheritance are shared with those of T. brucei mitochondria, where a single organelle partitions by association with the basal bodies of the two daughter flagella (53). Such similarities in mitosomal and mitochondrial biogenesis further suggest the evolutionary relatedness between these organelles.

The absence of a remnant organelar genome in mitosomes has thus far prevented a direct genetic demonstration of their mitochondrial ancestry, but the reported presence of DNA in the mitosomes of Blastocystis is a promising development (4, 5, 54, 55). The genetic demonstration that Nyctotherus hydrogenosomes are modified mitochondria suggests that reductive evolution of the original mitochondrial endosymbiont may have given rise to aerobic and anaerobic mitochondria, hydrogenosomes, and, in all likelihood, to mitosomes as predicted by the hydrogen hypothesis for the first eukaryote (5, 56–58). The confirmed cellular localization of mitochondrial marker proteins in Giardia mitosomes, their functionally conserved protein import mechanisms, as well as their conserved features of organelle replication and inheritance are all evidence that Giardia mitosomes represent vestigial anaerobic mitochondria.

Acknowledgments—We thank the Giardia Genome Project for making sequence data available before publication. We thank Drs. A. Schneider and M. Marti for critical reading of the manuscript, Drs. A. Harvey and M. Crompton for help with mammalian cell transfections, and T. Michel and N. Sommerville for excellent technical assistance.

REFERENCES
1. Williams, R. A., Hirt, R. P., Lucoq, J. M., and Emlrey, T. M. (2002) Nature 418, 865–869
2. Riordan, C. E., Ault, J. G., Langreth, S. G., and Keithly, J. S. (2003) Curr. Genet. 44, 138–147
3. Tovar, J., León-Avila, G., Sanchez, L. B., Sutak, R., Tachczy, J., van der Giezen, M., Hernandez, M., Müller, M., and Lucoq, J. M. (2003) Nature 426, 172–176
4. León-Avila, G., and Tovar, J. (2004) Microbiology 150, 1245–1250
5. Nastrudene, A. M., and Tan, K. S. (2004) J. Microbiol. Methods 58, 101–109
6. Müller, M. (2003) in Molecular Medical Parasitology (Marr, J., Nilsen, T., and Komuniecki, R., eds), pp. 125–139, Academic Press, London
7. Roger, A. J., and Silberman, J. D. (2002) Nature 418, 827–829
