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Evolution

A common evolutionary origin for mitochondria and hydrogenosomes

(symbiosis/organelle/anaerobic protist)

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ABSTRACT **Trichomonads are among the earliest eukaryotes to diverge from the main line of eukaryotic descent. Keeping with their ancient nature, these facultative anaerobic protists lack two "hallmark" organelles found in most eukaryotes: mitochondria and peroxisomes. Trichomonads do, however, contain an unusual organelle involved in carbohydrate metabolism called the hydrogenosome. Like mitochondria, hydrogenosomes are double-membrane bounded organelles that produce ATP using pyruvate as the primary substrate. Hydrogenosomes are, however, markedly different from mitochondria as they lack DNA, cytochromes and the citric acid cycle. Instead, they contain enzymes typically found in anaerobic bacteria and are capable of producing molecular hydrogen. We show here that hydrogenosomes contain heat shock proteins, Hsp70, Hsp60, and Hsp10, with signature sequences that are conserved only in mitochondrial and α-Gram-negative purple bacterial Hsps. Biochemical analysis of hydrogenosomal Hsp60 shows that the mature protein isolated from the organelle lacks a short, N-terminal sequence, similar to that observed for most nuclear-encoded mitochondrial matrix proteins. Moreover, phylogenetic analyses of hydrogenosomal Hsp70, Hsp60, and Hsp10 show that these proteins branch within a monophyletic group composed exclusively of mitochondrial homologues. These data establish that mitochondria and hydrogenosomes have a common eubacterial ancestor and imply that the earliest-branching eukaryotes contained the endosymbiont that gave rise to mitochondria in higher eukaryotes.**

The origin of the hydrogenosome, an enigmatic organelle found exclusively in eukaryotes that lack mitochondria, has been a topic of much debate (1–7). The hydrogenosome was first described (8, 9) and has been most extensively analyzed in trichomonads (4). Hydrogenosome-like organelles have also been identified in a broad phylogenetic range of organisms, including rumen-dwelling ciliates (10–12) and fungi (13, 14) as well as free-living ciliates (3). In addition to lacking mitochondria, organisms that contain hydrogenosomes also share the feature of being facultative anaerobes.

Hydrogenosomes are the site of pyruvate fermentation and play a central role in carbohydrate metabolism in trichomonads. Within the organelle, pyruvate is broken down to acetate, CO_2, and molecular hydrogen. This process is coupled to ATP formation via substrate-level phosphorylation. Biochemical analyses of hydrogenosomes have revealed properties that are similar to those of mitochondria; however, there are significant differences. For example, the enzyme that mediates decarboxylation of pyruvate in hydrogenosomes, pyruvate/ferredoxin oxidoreductase, is markedly different from its counterpart in mitochondria, the pyruvate dehydrogenase complex. Likewise, mitochondria do not possess a hydrogenase, a marker enzyme of the hydrogenosome, nor do they produce molecular hydrogen. Pyruvate/ferredoxin oxidoreductase and hydrogenase are, in contrast, commonly found in anaerobic bacteria. Like mitochondria, hydrogenosomes are bounded by a double membrane (15); however, the inner membrane neither forms cristae nor contains detectable cytochromes or cardiolipin as found in mitochondria (16, 17). Also, hydrogenosomes do not appear to contain F0F1 ATPase activity (18). On the other hand, ATP is produced in hydrogenosomes via catalysis by succinyl CoA synthetase (19, 20), a Krebs cycle enzyme that catalyzes the same reaction in hydrogenosomes and mitochondria.

To determine whether hydrogenosomes share a common origin with mitochondria or evolved independently of mitochondria, we have conducted biochemical and phylogenetic analyses on heat shock proteins Hsp70, Hsp60, and Hsp10 of the trichomonad, *Trichomonas vaginalis*. Phylogenetic analyses using Hsp70 and Hsp60 have previously confirmed that mitochondria are endosymbiotic descendants from α-Gram-negative purple bacteria (21, 22). The data reported here show that a common endosymbiont gave rise to both mitochondria and hydrogenosomes.

MATERIALS AND METHODS

Cells. Trichomonads were cultured, and whole cell extracts, cytosolic fractions, and purified hydrogenosomes were prepared as described (19, 20).

Isolation and Characterization of cDNA and Genomic Clones. *T. vaginalis* Hsp70 cDNA clone was isolated by screening an expression library with polyclonal antisera made against purified hydrogenosomes, as described (23). A *T. vaginalis* Hsp70 genomic clone was isolated from a library constructed in λ Zap (19) with the cDNA as a probe following standard procedures. Hsp60 and Hsp10 sequences were cloned by polymerase chain reaction (PCR) with *T. vaginalis* genomic DNA as template. Degenerate primers sets 5′-GC(TC)GG-(TC)GG(TC)CCAGG(TC)AAGGG(TC)ATG-3′ and 5′-AC-TGG(AG)ATCTT(AG)CG(AG)CC(AG)TG-3′, and 5′-TC-(ACT)GG(CT)AT(CT)GT(CT)AT(CT)CCA-3′ and 5′-C-(AG)AC(AG)AT(AG)GC(AG)AG(AG)AT(AG)TC-3′ were used to generate 330-bp and 273-bp fragments encoding parts of Hsp60 and Hsp10, respectively. The cloned PCR products were used as probes to obtain genomic clones containing the entire gene. The genes were sequenced according to the Sanger method (Sequenase 2.0 kit; United States Biochemical).

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Abbreviation: Hsp, heat shock protein

Data deposition: The sequences reported in this paper have been deposited in the GenBank data base [accession nos. U27231 and U27232 (*Trichomonas vaginalis* Hsp70), U26966 (*T. vaginalis* Hsp60), and U26965 (*T. vaginalis* Hsp10)].

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Immunoblot Analysis. *T. vaginalis* whole cell extracts, cytosolic fractions, and hydrogenosomes were isolated as described (20). Lysates were prepared from untransformed BL21 cells and from cells harboring the Hsp70 cDNA clone induced to express the clone in the presence of 1 mM isopropyl β-D-thiogalactoside, according to standard procedures. Protein (10 μg) for each *T. vaginalis* subcellular fraction or *Escherichia coli* lysate was analyzed by SDS/PAGE and immunoblotting, as described (20).

Protein Purification and Amino Terminal Sequencing. *T. vaginalis* Hsp60 was isolated using ATP agarose by a modified procedure (24). Purified hydrogenosomes (5 mg of protein) were resuspended in 1 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), 50 μg of N^{α}-(*p*-tosyl)lysine chloromethyl ketone per ml, and 10 μg of leupeptin per ml, and sonicated on ice five times for 10 sec, each with 1-min intervals until the solution was cleared. Lysed organelles were then centrifuged at 2500 × *g* for 5 min at 4°C, and the supernatant was recentrifuged at 8000 × *g* for 30 min at 4°C. The resulting supernatant was adjusted to 0.5 M KCl, 0.5 M NaCl, and 5 mM $MgCl_2$. ATP-agarose (A2767; Sigma) was equilibrated with buffer B (50 mM Tris, pH 8.0/1 mM EDTA/0.2 mM PMSF/0.5 M KCl/0.5 M NaCl/5 mM $MgCl_2$) (24) and proteins in the supernatant were mixed with the agarose beads for 15 h at 4°C, inverting end-to-end. The matrix was washed with 20 bed volumes of buffer B and eluted by increasing the [ATP] in buffer B from 5 to 15 mM. Proteins eluted at 10 mM ATP were subjected to 10% SDS/PAGE and immunoblot analysis using the Cyanobacteria (StressGen Biotechnologies, Sidney, Canada) Hsp60 antisera to verify the presence of a cross-reacting 60-kDa protein. The proteins were then concentrated, size-separated by 10% SDS/PAGE, and electroblotted onto a polyvinylidine difluoride filter (Bio-Rad) according to conditions recommended by the manufacturer. The 60-kDa band was excised and subjected to microsequencing analysis by Edman degradation in the Biological Chemistry Microsequencing Facility, University of California, Los Angeles, School of Medicine.

Phylogenetic Analysis. Evolutionary trees were generated with the use of PHYLIP 3.5c (25). A Fitch Majority-Consensus tree was obtained after bootstraps and distance matrix sets were generated using PROTDIST. Trees were drawn with the DRAWGRAM program. Phylogenetic analyses of Hsp70 and Hsp10 were carried out on the sequence corresponding to *T. vaginalis*, amino acids 39-655 and 13-107, respectively. Amino acid sequences of Hsp10 could be aligned in all species without significant gaps, extensions, or deletions. Minor gaps and extensions in aligned Hsp70 sequences were deleted. The analysis of Hsp60 was carried out on a sequence corresponding to *T. vaginalis*, amino acids 12-470, plus an additional 98 amino acids found at the carboxyl termini of all Hsp60s except the *T. vaginalis* protein. Other than the ≈98 amino acid C-terminal deletion on the *T. vaginalis* proteins, all Hsp60s were aligned without significant gaps, extensions, or deletions.

RESULTS AND DISCUSSION

As heat shock proteins are well suited for determining phylogenetic relationships (26, 27), we have analyzed Hsp70, Hsp60, and Hsp10 from *T. vaginalis*, a parabasalid that contains hydrogenosomes, to address the origin of this organelle. Hsp70 is ubiquitously found in eubacteria, archaebacteria, eukaryotic organelles, and cytosol, whereas Hsp60 and Hsp10 are present in eubacteria and eukaryotic organelles, but not in archaebacteria or eukaryotic cytosol. We examined whether *T. vaginalis* hydrogenosomes contain Hsp homologues by screening an expression library (23) with polyclonal antisera that react specifically with hydrogenosomal proteins (Fig. 1*A*). Of several clones analyzed, a 1.7-kb cDNA encoding Hsp70 was selected. Immunoblot analysis was performed to confirm that

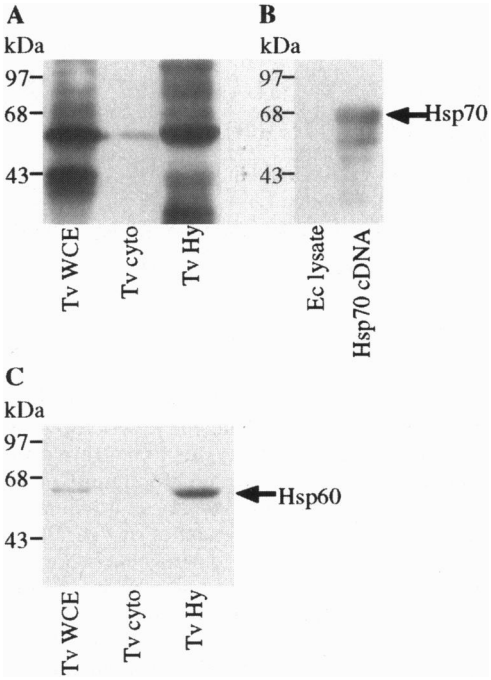

FIG. 1. *T. vaginalis* Hsp70 and Hsp60 are localized in the hydrogenosome. *T. vaginalis* subcellular fractions and bacterial lysates prepared from nontransformed *E. coli* strain BL21 and transformed *E. coli* expressing a trichomonad Hsp70 cDNA clone were size separated on 10% SDS/PAGE, electroblotted to nitrocellulose, and reacted with either antisera generated against total purified hydrogenosomes (*A* and *B*) or antisera against Hsp60 from *Cyanobacteria* (StressGen Biotechnologies) (*C*). Tv WCE, *T. vaginalis* whole cell extract; Tv cyto, *T. vaginalis* crude cytosolic fraction; Tv Hy, *T. vaginalis* purified hydrogenosomes; Ec lysate, untransformed *E. coli* strain BL21 lysate; Hsp70 cDNA, lysate of *E. coli* expressing TvHsp70 cDNA clone. Size markers are in kilodaltons.

the protein encoded by the cDNA is found in the organelle (Fig. 1*B*). We also tested whether Hsp60 is present in hydrogenosomes by reacting blots containing *T. vaginalis* whole cell extracts, cytosolic fractions, and purified hydrogenosomes with antisera against Cyanobacteria Hsp60. The fact that the antisera react with purified hydrogenosomes and not with cytosolic fractions (Fig. 1*C*) shows that *T. vaginalis* Hsp60 is specifically localized to hydrogenosomes.

Hsp60 and Hsp10 genes were isolated by degenerate PCR. These PCR products and the Hsp70 cDNA were then used as probes to isolate *T. vaginalis* genomic clones from which we determined the complete coding sequence of all three genes (data not shown; sequences available through GenBank, see Table 1). The deduced amino acid sequences predict proteins of 655, 470, and 107 amino acids for Hsp70, Hsp60, and Hsp10, respectively. Southern blot analyses using *T. vaginalis* genomic DNA reveals that each of the three organellar Hsps is encoded by a single gene (data not shown).

The N-terminal sequence of Hsp60 purified from *T. vaginalis* hydrogenosomes begins 14 amino acids from the initiating methionine (Fig. 2). This indicates that the protein is synthesized with a presequence that is cleaved from the mature protein found in the organelle. Amino terminal, cleavable sequences have also been observed for other hydrogenosomal proteins (4, 19, 23, 31, 32). Our preliminary data indicate that these presequences are necessary for translocation of proteins into the hydrogenosome, similarly to what has been observed for translocation of proteins into mitochondria (P.J.B. and P.J.J., unpublished results).

A comparison of the predicted amino acid sequence of hydrogenosomal Hsps with eubacterial and eukaryotic homologues, by BESTFIT analysis, shows extensive sequence similarity

Table 1. Species names and accession numbers for Hsp70, Hsp60 and Hsp10 genes

Species	Hsp70	Hsp60	Hsp10
Hydrogenosome			
T. vaginalis	U27232	U26966	U26965
	U27231		
Eukaryotic cytosol:			
Bos taurus	U09861		
Bugia malayi	P27541		
Chlamydomonas reinhardtii	P25840		
Daucus carota	P26791		
Drosophila melanogaster	P02825		
Entamoeba histolytica	M84652		
Giardia lamblia	U04874		
Hamster	P19378		
Human	P08107		
Leishmania major	P14834		
Pisum sativum	Z32537		
Saccharomyces cerevisiae	P11484		
Schistosoma mansoni	P08418		
Trypanosoma cruzi	P05456		
Endoplasmic reticulum			
Giardia lamblia	U04875		
Human	P11021		
Spinacia oleracea	L23551		
Soybean	U08384		
Saccharomyces cerevisiae	P16474		
Saccharomyces pombe	P36604		
Trypanosoma brucei	L14477		
Mitochondria			
Bos taurus			X69556
Cucurbita sp. (pumpkin)		X70868	
Hamster		P18687	
Human	L15189	P10809	X75821
Leishmania major	X64137		
Saccharomyces cerevisiae	P12398	P19882	X75754
Saccharomyces pombe	P22774		
Trypanosoma cruzi	P20583	L08791	
Zea mays		P29185	
Chloroplasts			
Arabidopsis thaliana		P21240	
Brassica napus α		P21239	
Brassica napus β		P21241	
Cryptomonas phi	P29215		
Pisum sativum	L03299		
Spinacia oleracea			Q02073
Triticum aestivum (wheat)		P08823	
Cyanobacteria			
Synechococcus sp. 6301			P07889
Synechococcus sp. 6803		P22034	
Synechococcus sp. 7942			P22880
Chlamydia and spirochaetes			
Chlamydia trachomatis	L22180	P17203	P17204
Leptospira interrogans		P35468	P35472
Gram-negative bacteria			
Agrobacterium tumefaciens		P30779	
Bradyrhizobium japonicum		P35862	
Brucella abortus		P25967	P25968
Coxiella burnetti		P19421	P19422
Escherichia coli	K01298	P06139	
Haemophilus ducreyi		P31294	
Neisseria gonorrhoeae		P29842	
Pseudomonas aeroginosa			P30720
Rhizobium meliloti		P35471	M94192
Yersinia enterocolitica			D14078
Archaebacteria and Gram-positive bacteria			
Bacillus subtilis			M81132
Clostridium acetobutylicum			M74572
Clostridium perfringens	X62915	P26821	M94192
Haloarcula marismortui	M84006		
Mycobacterium leprae		P09239	
Sulfolobus shibatae (tcp-1)		P28488	
Staphylococcus aureus			D14175
Streptomyces albus			P26822
Thermophilic bacteria		P26209	S57424
Thermoplasma acidophilum	L35529		
Mycobacterium tuberculosis			X60350

among these proteins. Sequence similarity between hydrogenosomal Hsp70, Hsp60, and Hsp10 and their homologues in other organisms ranges from 60.2 to 70%, 63.5 to 74.1%, and 51.4 to 62.6%, respectively. To address the similarity between hydrogenosomal and other organellar Hsps, we looked for "signature sequences" that have been used to classify Hsps (21, 22). Strikingly, hydrogenosomal Hsp70 and Hsp60 both possess signature sequences previously observed exclusively in mitochondrial and α-Gram-negative purple bacterial homologues (Fig. 3 *A* and *B*). Hydrogenosomal Hsp10 also contains a number of amino acids that are conserved in mitochondrial Hsp10s, but not in others (Fig. 3*C*).

As a further test of a common ancestry for hydrogenosomes and mitochondria, we performed phylogenetic analyses on hydrogenosomal Hsps using 34 different Hsp70s, 25 Hsp60s, and 21 Hsp10s (Table 1). Fig. 4 shows the results of analyses obtained using the PHYLIP 3.5c program (25). Sequences aligned by PILEUP were bootstrapped 1000 times, protein distance matrices were determined, and phylogenies were constructed according to the Fitch program (Fig. 4). Alternative treeing algorithms, including neighbor–joining and PAUP programs (33), yield identical topologies (data not shown). Moreover, the topology of the resulting trees is the same as that previously reported for Hsp homologues (21, 22). These analyses show that hydrogenosomal proteins Hsp70 and Hsp60 branch 99% of the time with a monophyletic group formed exclusively by mitochondrial homologues (Fig. 4 *A* and *B*). Similarly, hydrogenosomal Hsp10 groups with mitochondrial homologues 62% of the time (Fig. 4*C*). The long branch length of hydrogenosomal Hsp70 may result from unequal rates of amino acid replacement, leading to long branch attraction (34, 35). This unequal rate effect has been noted for domains of different Hsp70s (36). In contrast, the branch length of both hydrogenosomal Hsp60 and Hsp10 do not appear to be affected by unequal rate effects. Moreover, the probability of all three hydrogenosomal Hsps branching with mitochondrial homologues simply by chance is essentially zero. Thus, the data shown in Fig. 4 demonstrate that hydrogenosomal and mitochondrial Hsps have a common eubacterial ancestor.

It was originally proposed that hydrogenosomes originated through the endosymbiotic adoption of an anaerobic bacterium by a primitive eukaryotic cell, in view of the ability of the organelle to metabolize in the absence of oxygen (5, 7). An opposing hypothesis, which took into account certain structural and biochemical similarities between hydrogenosomes and mitochondria, proposed that hydrogenosomes are derived from mitochondria (1, 2). Recent evolutionary analyses of ribosomal RNA (rRNA) sequences have demonstrated that trichomonads diverged from the main line of eukaryotic evolution prior to the advent of authentic mitochondria (37–40). In the present study, we show that hydrogenosomes contain Hsps and that hydrogenosomal Hsp60 has a cleaved leader sequence similar to mitochondrial import sequences. Thus, hydrogenosomes and mitochondria appear to have similar protein import mechanisms, as predicted if the two organelles evolved from a common ancestor (41). Acquisition

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T.vaginalis(Hy)                                MSL IEAAKHFTRA FAKARDLKFG SDARDHLL.L .GVEKLADAV
Pumpkin(Mito)         MHRFATGLAS KARLARNGAN QIASRSNW-R NYA-K-V--- VE--GL..M- K---D-----
Human(Mito)                MLRLP TVFRQMRPVS RVL-P-L--- Y---V..--- A---ALM-Q. .--DL-----
E.coli                                                   MA-K-V--- N---VKM-R. .--NVL----
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FIG. 2. *T. vaginalis* Hsp60 purified from hydrogenosomes lacks a 14-amino acid N-terminal sequence that is encoded in the gene. Hsp60 was isolated from purified hydrogenosomes (19) and subjected to N-terminal sequence analysis. The first 27 residues derived from amino acid sequencing (AKARDLKFGSDARDHLLLGVEKLADAV) match amino acids 15-41 encoded in the gene, showing that the mature protein lacks 14 amino acids at the N terminus. Previously reported N-terminal sequences identified for Hsp60 isolated from mitochondria of pumpkin (*Cucurbita sp.*, GenBank accession no. X70868) (28) and human (*Homo sapiens*, GenBank accession no. P10809) (29) and the presequences of these proteins, as well as the N terminus of *E. coli* Hsp60 (GenBank accession no. P06139) (30) are shown for comparison. The N-terminal sequences derived for *T. vaginalis*, pumpkin, and human Hsp60 are underlined. Hy, hydrogenosomal; Mito, mitochondrial. A hyphen indicates the amino acid is identical to that in the *T. vaginalis* protein, and period indicates a gap in the protein sequence.

of protein import machinery is a trait derived after establishment of an endosymbiont with a host cell; thus, the presence of similar import mechanisms argues against independent symbiotic origins. Moreover, our phylogenetic analyses show that mitochondrial and hydrogenosomal Hsps are closely related. These data strongly support the hypothesis that hydrogenosomes and mitochondria have a common eubacterial ancestor and have evolved from the same progenitor organelle. We propose that this progenitor organelle gave rise to mitochondria in the aerobic niches occupied by higher eukaryotes and to hydrogenosomes in the anaerobic niches colonized by trichomonads. Unlike mitochondria, hydrogenosomes do not contain genetic material (42); however, as most genes in the mitochondrial genome encode respiratory chain proteins (43) that are absent in hydrogenosomes (42), the maintenance of a hydrogenosomal genome seems unnecessary.

It has long been known that certain early-diverging protists (i.e., kinetoplastids) contain a highly-modified mitochondrion, called the kinetoplast, that is both biochemically and structurally different from mitochondria found in higher eu-

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A) Hsp70:               71  75    109    116   325              336
T.vaginalis(Hy)         IQN AE    TFF AT KRL   PFI TVTG ..AG PKH
Human(Mito)             LE- --    --Y -- ---   --- -MDS ..S- ---
S.cerevisiae(Mito)      -E- --    -L- -- ---   --- -ADA ..S- ---

Human(C)                -A- DQ    -V- DA ---   ... ..DS L YE- ...
S.cerevisiae(C)         -P- EL    -I- DV ---   ... V-DS L ID- ...
Hamster(C)              -A- DQ    -V- DA ---   ... ..DS L FE- ...
D.carota(C)             -A- DQ    -V- DA ---   ... ..DS L YE- ...
T.cruzi(C)              -A- DQ    -V- DA ---   ... ..DS L FE- ...

B) Hsp60:               160                   179    394            407
T.vaginalis(Hy)         NVAT I SA NG SEKIGH L IADA   SVG G A N E V E V GE EK
S.cerevisiae(Mito)      Q--- - -- -- DSHV-K - L-S-   R-- - - S - - - - -- --
Human(Mito)             Q--- - -- -- DKE--N I -S--   K-- - T S D - - - N- --
Hamster(Mito)           Q--- - -- -- DKD--N I -S--   K-- - T S D - - - N- --
Z.mays(Mito)            Q-G- - -- -- ERE--E - --K-   KI- - - S - A - - -- --
T.cruzi(Mito)           Q--- - -- -- D-EL-R - -GQ-   K-- - - G S E - - N- --

Wheat(Chl)              A--S - -- GN D-L--A M----    K-- A - T - T - L ED RQ
A.thaliana(Chl)         D--A V -- GN NDE--N M--E-    Q-- A Q T - T - L K- K-
B.napusα(Chl)           A--- - -- GN D-LV-T M--E-    K-- A - T - T - L ED R-
B.napusβ(Chl)           D--A V -- GN NAE--S M--E-    Q-- A Q T - T - L K- K-

C) Hsp10:               32          39   50        59
T.vaginalis(Hy)         KQKV.. G NL Y    QA TVIA V GPG
Human(Mito)             ETVT.K - GI M    -- --V- - -S-
S.cerevisiae(Mito)      -TAS.. . G- -    -- E-V- - -P-

C.acetobutylicum(G+)    EETT.K S FI V    M- E-V- - ---
M.tuberculosis(G+)      ETTT.A S G- V    EG --V- T ---
T.Bacteria(G+)          EE-T.A S GI V    EG R-V- - -A-
S.aureus(G+)            E-TT.K S GI V    EG VIV- - AT-
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FIG. 3. Hydrogenosomal Hsp70, Hsp60, and Hsp10 contain signature sequences found in mitochondrial homologues. Representative regions of Hsp70 (*A*), Hsp60 (*B*), and Hsp10 (*C*) that contain amino acids that are specifically conserved in particular groups of eubacterial or eukaryotic homologues are shown. For the sake of brevity, only signature sequences for mitochondrial Hsps and one other group of Hsp homologues are presented; however, the comparison used to identify these sequences included Hsps from all reported groups, aligned by PILEUP. Boxed residues denote conserved sequences that are found only in the class of Hsp indicated. The numbers at the top refer to amino acid positions in the *T. vaginalis* protein. A dash indicates the amino acid is identical to that in the *T. vaginalis* protein and a period indicates a gap in the protein sequence. Hy, hydrogenosome; Mito, mitochondria; Chl, chloroplast; C, cytosol; and G+, Gram-positive bacteria. Refer to Table 1 for accession numbers.

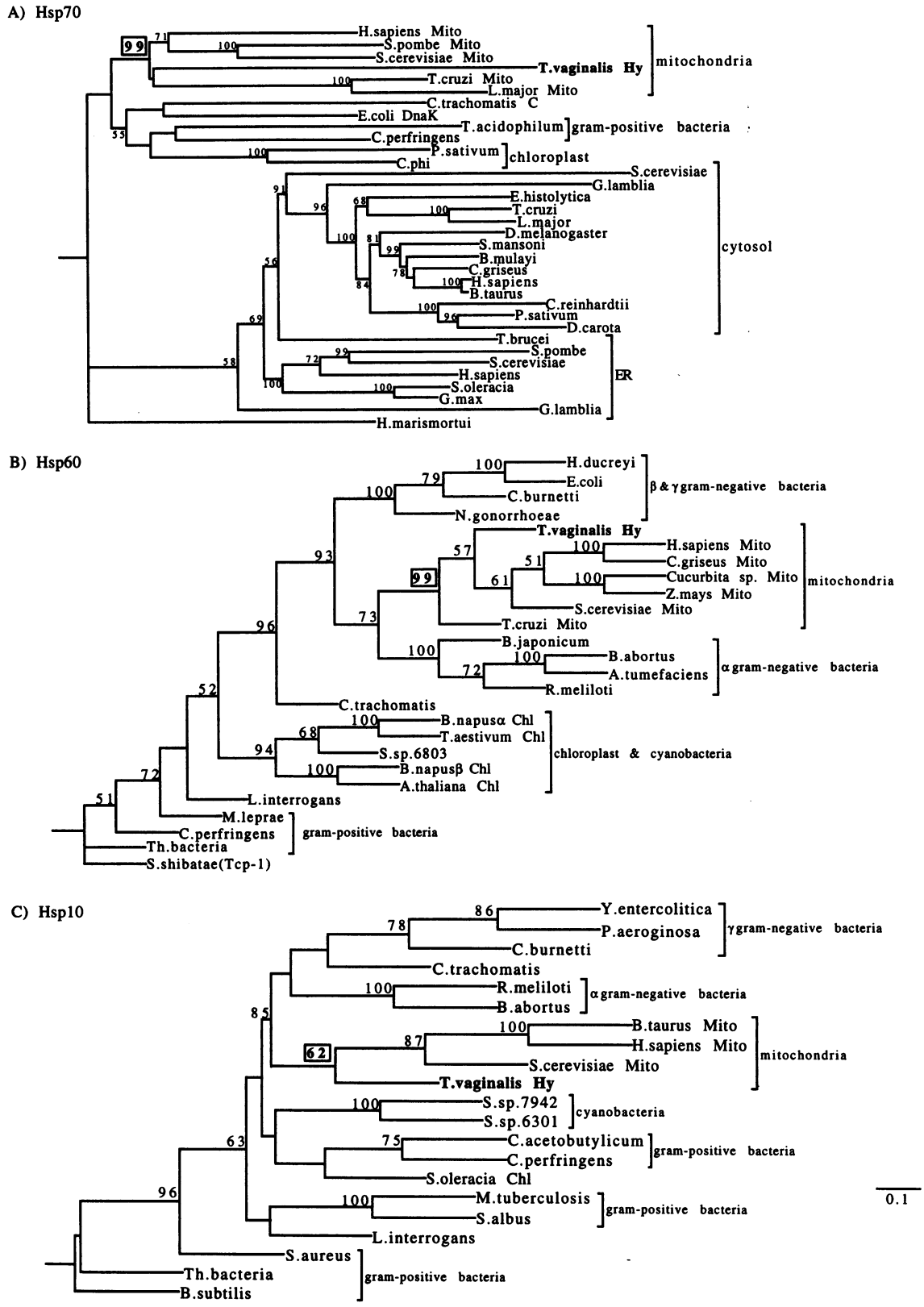

FIG. 4. Hydrogenosomal Hsps are phylogenetically related to mitochondrial homologues. Phylogenetic trees were derived for Hsp70 (*A*), Hsp60 (*B*), and Hsp10 (*C*). The numbers at the forks indicate the percentages of times that the species to the right of the fork grouped together (so-called bootstrap values) based on the generation of 1000 trees. Bootstrap values of less than 50% have been omitted. Horizontal branch lengths indicate the distances between species. Trees are unrooted except Hsp60, which is rooted using a distant relative Tcp-1 from *Sulfolobus shibatae* (22). The scale bar denotes 0.1 substitutions per amino acid. C, cytosol; ER, endoplasmic reticulum; Mito, mitochondria; Hy, hydrogenosome; Chl, chloroplasts. Refer to Table 1 for identification of the species used in this analysis and GenBank accession numbers.

karyotes. Our data show that three distinct organelles, hydrogenosomes, kinetoplasts, and mitochondria, are phylogenetically related. These results imply that the earliest-branching eukaryotes contained the endosymbiont that gave rise to

mitochondria in higher eukaryotes. It should be noted, however, that the possibility that the similar Hsps found in these three organelles are the remnants of lateral gene transfer by a different, transient, eubacterial symbiont cannot be strictly precluded.

Membrane-bounded organelles with hydrogenase activity have been identified in a broad range of amitochondriate organisms, including rumen-dwelling ciliates and fungi (6). In addition to these symbiotic organisms, hydrogenosome-like organelles are also found in free-living ciliates (3). The patchy distribution of these organelles and their structural diversity suggest a polyphyletic origin (1). However, the paucity of biochemical data on the organelles found in ciliates and fungi makes it difficult to determine the precise relationship between these organelles and trichomonad hydrogenosomes. Whether the shared origin reported here for *T. vaginalis* hydrogenosomes and mitochondria also applies to hydrogenosome-like organelles remains to be determined.

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