Hypothesis

Evolutionary relationship of Rickettsiae and mitochondria

Victor V. Emelyanov*

Department of General Microbiology, Gamaleya Institute of Epidemiology and Microbiology, Moscow 123098, Russia

Received 7 May 2001; accepted 30 May 2001

First published online 26 June 2001

Edited by Matti Saraste†

Abstract Phylogenetic data support an origin of mitochondria from the α-proteobacterial order Rickettsiales. This high-rank taxon comprises exceptionally obligate intracellular endosymbionts of eukaryotic cells, and includes family Rickettsiaceae and a group of microorganisms termed *Rickettsia*-like endosymbionts (RLEs). Most detailed phylogenetic analyses of small subunit rRNA and chaperonin 60 sequences consistently show the RLEs to have emerged before Rickettsiaceae and mitochondria sister clades. These data suggest that the origin of mitochondria and Rickettsiae has been preceded by the long-term mutualistic relationship of an intracellular bacterium with a pro-eukaryote, in which an invader has lost many dispensable genes, yet evolved carrier proteins to exchange respiration-derived ATP for host metabolites as envisaged in classic endosymbiont theory. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Phylogeny; Rickettsiaceae; *Rickettsia*-like endosymbiont; Mitochondrial ancestor; Mitochondrion; Hydrogenosome

1. Introduction

The endosymbiont theory for the origin of mitochondria is now widely accepted. As usually described, this theory states that an organelle traces its descent to a free-living bacterial ancestor that once entered into an endosymbiotic relationship with an ill-defined, primitively amitochondriate cell (pro-eukaryote). In the course of this relationship, most of symbiont genes have been lost or passed to the host genome. The result for invader was to become a modern mitochondrion, the main ATP-producing organelle of eukaryotes with a highly reduced genome [1–3]. Several new hypotheses have recently been advanced for the origin of mitochondria [4–7]. They are, however, inescapably not free of more or less plausible assumptions. Notably, free-living eubacterium often figures in the proposed scenarios as a candidate for mitochondrial progenitor [8].

Evolutionary processes involving primitive cells and organelles may be documented after employing a variety of phylogenetic methods [8–10]. Comparative studies of mitochondrial genomes unequivocally pointed to a eubacterial ancestry of mitochondria [2,8]. Their monophyletic nature and close relationship to the order Rickettsiales of α-Proteobacteria emerged from multiple phylogenetic reconstructions based on conserved proteins and small subunit (SSU) rRNA [7,11–16]. Due to the scarcity of relevant molecular information, above phylogenetic studies involved mostly aerobically respiring mitochondria and a few rickettsial species. It is known, however, that some primitive eukaryotes have anaerobic mitochondria [17,18] while others instead possess hydrogenosomes – energy-generating organelles which are suggested to be biochemically modified mitochondria [19,20]. Thus, comprehensive phylogenetic data are needed both to identify the nearest relatives of organelles and to answer the question of whether various mitochondria and hydrogenosomes share common ancestry. A phylogenetic analysis of chaperonin 60 (Cpn60), likely the best tracer of the eubacterial origin of organelles, has recently been published which involved all the rickettsial sequences known to date. This analysis demonstrated paraphyletic nature of the Rickettsiales and the closest relationship of the genus *Rickettsia* to mitochondria [16]. It is believed that a careful analysis of the nearest relatives of organelles, via involvement of additional molecular data, may give an insight into biological context of mitochondrial origin.

In this review I present phylogenetic evidence which supports a common origin of Rickettsiae and mitochondria from a highly reduced endosymbiotic bacterium. Based on these data, the probable nature of mitochondrial ancestor and molecular basis of obligate rickettsial symbiosis are considered.

2. Rickettsiae

Sequencing the *Rickettsia prowazekii* genome revealed striking similarity in the functional profiles of its genes to those of aerobic mitochondria [13]. This obligate intracytoplasmic bacterium is a typical representative of the genus *Rickettsia*, the basal group within the family Rickettsiaceae. Along with Rickettsiaceae, the order Rickettsiales includes some other α-proteobacterial species which are also non-cultivable in axenic medium. Thus, features of the various members of this large taxonomic group are of importance to understanding their relationship to mitochondria.

2.1. Taxonomy of the order Rickettsiales

Definition of Rickettsiae (the microorganisms of the order Rickettsiales) is a simple one. Rickettsiae are obligate intracellular endosymbionts and parasites of eukaryotic cells clas-

*Fax: (7)-095-193 61 83.
E-mail: 1570.g23@g23.relcom.ru

Abbreviations: AAC, ATP/ADP carrier; Cpn60, chaperonin 60; CA, common ancestor; LSU rRNA, large subunit rRNA; PFO, pyruvate:ferredoxin oxidoreductase; RLE, *Rickettsia*-like endosymbiont; SSU rRNA, small subunit rRNA

0014-5793/01/$20.00
PII: S0014-5793(01)02618-7

sified into α-subdivision of Proteobacteria [21,22]. It is now evident that the Rickettsiales consists of only the family Rickettsiaceae and a few bacteria collectively termed *Rickettsia*-like endosymbionts (RLEs) [22]. The Rickettsiaceae comprise an enormous variety of the species which are, for the most part, commensals of arthropods. The family is represented by the tribes Rickettsiae, Wolbachiae, polyphyletic Ehrlichiae, and some *Anaplasma* species [22–24]. The tribe Rickettsiae includes the genera *Rickettsia* and *Orientia*, the latter being represented by several strains of the single species *O. tsutsugamushi* [25]. Genus *Rickettsia* subdivides into typhus group (TG) and spotted fever group (SFG), but also includes several marginal members such as *Rickettsia bellii* [23]. TG is represented by two species, of which the most known is *R. prowazekii* – an etiological agent of louse-borne epidemic typhus. SFG comprises endosymbionts of ticks, with only few of them, e.g. *R. rickettsii*, occasionally infecting humans [23]. Curiously, two plant-associated Rickettsiae were recently described. Phylogenetic analyses consistently place them within the genus *Rickettsia* [26,27].

Diverse Ehrlichiae are known as parasites of ticks, mammals, trematodes, and even Saccamoebae [24,28]. Tribe Wolbachiae was recognized to be a vast group of species widely spread among insects but also found in nematodes [29]. Phylogenetic studies indicate that the species of Ehrlichiae intermingle with Wolbachiae and *Anaplasma marginale* (which is, in effect, an Ehrlichia) within a group separated from the tribe Rickettsiae [12,22,25]. The Rickettsiaceae have recently been augmented by two species reported in amoeba *Acanthamoeba castellanii* [28].

A few RLEs known to date were found to exhibit a specific, yet distant relationship to Rickettsiaceae. These are *Holospora obtusa* and *Caedibacter caryophila* parasitizing different strains of the ciliate *Paramecium caudatum* [21], and an etiological agent of necrotizing hepatopancreatitis in shrimp (NHP agent) [30].

2.2. Biological features of Rickettsiae

It has long been recognized that the Rickettsiae are incapable of growth in any rich artificial medium [23]. *R. prowazekii* penetrates the target cell by a process termed induced phagocytosis, but quickly escapes from the phagosome into the cytosol. The nature of phospholipase, apparently involved in both entry and exit of parasite, is unknown to date. Regardless of whether it is a bacterial enzyme or Rickettsiae induce host phospholipase activity, manifestation of this activity during intimate contact of the parasite with the host [23] is thought to be fully consistent with their ability to grow only inside intact, living cells.

Ehrlichiae are known to multiply within parasitophorous vacuoles. Despite an apparent lack of cell cycle, they exist in two (light and dense) forms [24]. Whereas *Rickettsia* species are typical Gram-negative bacteria, both *Orientia* and Ehrlichiae were shown to be deficient in peptidoglycan and lipopolysaccharides [24,25]. Like *R. prowazekii* [13], Ehrlichiae are able to respire and lack glycolysis [24]. The species of the tribe Wolbachiae manipulate mitosis in insects causing sex ratio distortion [29]. Biological data suggest that the *Orientia*, Ehrlichiae and Wolbachiae are a diversification of the genus *Rickettsia*. Rickettsial genomes are rather small compared to those of free-living bacteria [24].

RLEs are poorly understood. *H. obtusa*, *C. caryophila* and NHP agent are known to be non-cultivable on bacteriological medium [21,30].

3. Mitochondria and hydrogenosomes

Energy metabolism is central to an issue of the origin and evolution of ATP-generating organelles and the eukaryotic cell itself. Modern eukaryotes (no photosynthetic organisms are considered) produce ATP in different routes ranging from cytoplasmic glycolysis and fermentation to compartmentalized respiration and hydrogen-evolving fermentation. Thus, relationship between mitochondria and hydrogenosomes is of high importance in the framework of endosymbiont theory.

3.1. Energy metabolism

A hallmark role of mitochondria is to produce ATP for numerous needs in the eukaryotic cell. This complex process normally starts in the matrix with pyruvate dehydrogenase-mediated oxidative decarboxylation of pyruvate to yield NADH and acetyl-CoA. The latter one then feeds TCA, generating most of the NADH. Electrons from NADH enter the inner membrane-located electron transport (respiratory) chain in which they are transferred from one respiratory complex to another, coupled with translocation of protons from matrix to intermembrane space. ATP synthase passes protons back into the matrix accompanied by ATP synthesis. Finally, ATP/ADP carrier (AAC) exchanges mitochondrial ATP for cytosolic ADP. Most higher eukaryotes use oxygen as terminal acceptor of electrons (for review, see [31]). In mitochondria of several primitive fungi, nitrogen oxides serve as alternative terminal electron acceptors [17]. Some flatworms and other primitive animals instead reduce fumarate to succinate in a reaction catalyzed by fumarate reductase [18]. Anaerobic respiration is well known to be widely spread among free-living α-Proteobacteria [17,18].

Some ciliates, parabasalia (e.g. *Trichomonas vaginalis*) and chytridiomycetes lack mitochondria, yet possess another type of energy-generating organelles called hydrogenosomes [10,20,32,33]. Conversion of pyruvate to acetyl-CoA is carried out in these organelles by pyruvate:ferredoxin oxidoreductase (PFO) [33,34]. Hydrogenase, a uniquely hydrogenosomal enzyme, reoxidizes reduced in the above reaction ferredoxin liberating molecular hydrogen. Hydrogenosomes lack respiratory chains (i.e. oxidative phosphorylation) and produce ATP via substrate level phosphorylation [4,20,33]. It is worth noting that secondarily amitochondriate protists such as *Entamoeba histolytica* and diplomonad *Giardia lamblia* also possess PFO, but produce ATP in the cytoplasm via fermentation without evolving H_2 [34]. Recent phylogenetic data point to a single eubacterial origin of eukaryotic PFOs, although bacterial sister group has escaped identification [10].

Mitochondria and hydrogenosomes are known to have a partially overlapping enzymatic content [20,33]. Of importance, both possess tightly related AAC [35]. Consistent with these facts, several lines of the data indicate that hydrogenosomes may be biochemically modified mitochondria (see below).

3.2. Biogenesis of organelles

The key process of mitochondrial biogenesis is known to be an import of proteins synthesized in the cytoplasm (reviewed in [36]). They are encoded by the genes which have been trans-

ferred from endosymbiont to the nucleus during mitochondrial evolution and serve functions mostly in bioenergetic and information transfer pathways [8]. To be targeted into organelle, nucleus-specified proteins must be tagged with cleavable or uncleavable targeting sequences, these having no conserved primary structure. Mitochondrial import machinery is very complex [36], with its evolutionary origin being a major enigma in the framework of endosymbiont theory. Along with import, mitochondria are also able to export some proteins particularly via ubiquitous Oxa1 [37]. A gene encoding its ortholog YidC, inner membrane protein, has been found in many bacteria including *R. prowazekii* [13].

Both targeting sequences and protein import machineries were recently reported to be interchangeable in aerobic mitochondria and hydrogenosomes [20,35,38]. The organelles also share some physico-chemical and morphological properties [33].

3.3. Mitochondrial genomics

Comparative genomics studies involving numerous mitochondrial genomes sequenced to date unequivocally show that aerobically respiring mitochondria have a single, eubacterial origin [2,8]. In particular, an unprecedented variety of eubacterial traits have been reported in the mitochondrial genome of freshwater zooflagellate (jakobid) *Reclinomonas americana* [8]. Gene flow from mitochondrial to nuclear genomes has been documented in some detail [2,39]. Studies on gene complement show that retention of mitochondrial genome may be driven by the only two genes, *cob* and *cox1*, specifying intensely hydrophobic, unimportable subunits of respiratory complexes III and IV, respectively [2,40].

Hydrogenosomes typically lack DNA [20]. Nonetheless, hydrogenosome of the ciliate *Nyctotherus ovalis* inhabiting hindgut of cockroaches was recently described which does possess a genome. Phylogenetic analysis, involving SSU rRNA encoded in this genome, groups the above ciliate with closely related but mitochondria-containing species [32,41].

3.4. Suggested relationship between mitochondria and hydrogenosomes

It is now becoming evident that the origin of mitochondrion-related organelles and of their enzymatic content should be considered separately [20]. I suggest that an organelle per se has originated once from RLE (see below). Enzymes of both aerobic and anaerobic respiration are thought to have arisen in the same single event. It seems unlikely that energy metabolism of anaerobic mitochondria has originated prior to an establishment of aerobic mitochondria, and components of anaerobic respiration, to say, waited for an appearance of aerobic mitochondria provisionally functioning e.g. in non-organellar membranes of host cell. In contrast, specific hydrogenosomal enzymes, such as PFO, might have a separate, more ancient origin. As in the case of above-mentioned amitochondriate protists, they could have been involved in cytosolic fermentation before an advent of organelle. Subsequent to this event, hydrogenosome-like proteins would be easily recruited to organelle merely upon acquisition of targeting sequence [15] and other rearrangements [32]. It is also suggested that anaerobic mitochondria and hydrogenosomes could not have been evolutionarily converted to aerobic mitochondria because former ones are unlikely to have specified unimportable proteins (Cob and Cox1) characteristic of latter ones. But the reverse seems to be true – aerobically respiring mitochondria could have been irreversibly converted to either anaerobic mitochondria or hydrogenosomes. The only requirement would be the presence in the nuclear genomes of the genes encoding respective enzymes. Indeed, PFO-like domains have recently been reported in several ascomycete fungi and protists [10]. *N. ovalis* hydrogenosome has been shown to import an unusual hydrogenase composed of the domains of several redox proteins [32]. Thus, the common ancestor (CA) of organelles might have been an aerobically respiring mitochondrion or even a sort of endosymbiont. Conversion of mitochondria to hydrogenosomes in some unicellular eukaryotes is considered as an adaptation to life with little or no oxygen [20].

4. Closest rickettsial relatives of mitochondria and hydrogenosomes

Phylogenetic studies are crucial to an establishment of the closest extant relatives of organelles. Perhaps the main purpose that they serve is to help in a better choice of a bacterium for detailed molecular analysis. Together, the results of such efforts may provide an excellent basis to hypothesize on the origin of mitochondria.

4.1. Phylogenetic data

First phylogenetic data, based on the use of most well represented Cpn60 amino acid sequences and SSU rRNA nucleotide sequences, have consistently shown that the monophyletic cluster of aerobic mitochondria emanates from the order Rickettsiales [11,12]. *R. prowazekii* genome [13] provided ample information for phylogenetic reconstructions. Concatenated sequences of ribosomal proteins and protein sequences of respiratory complexes [13,14] strongly supported a relationship of mitochondria and Rickettsiales. However, subsequent reanalysis of Cob and Cox1–3 has revealed that some free-living α-Proteobacteria and *R. prowazekii* may be equally close relatives of mitochondria [8]. Recent study of the yeast mitochondrial proteome has established that not all of ca. 50 proteins, which turned out to be of α-proteobacterial origin, point to a sisterhood of *R. prowazekii* and mitochondria [7]. In effect, some macromolecules may be inappropriate phylogenetic markers to resolve a close relationship. Apparently, large subunit (LSU) rRNA is not the case (Fig. 1A). One may complain that LSU rRNAs from RLEs have not been sequenced to date.

Albeit the use of SSU rRNA in phylogenetic studies has some limitations [11,12], a great number of sequences from Rickettsiales are presently known, thus allowing the most explicit analysis. SSU rRNA-based maximum likelihood (ML) tree involving diverse species of Rickettsiaceae and all RLEs for which the sequences are available is shown in Fig. 1B. In addition to mitochondrial sequences of higher plants and *R. americana*, recently published SSU rRNA sequence from *N. ovalis* hydrogenosome [41] together with mitochondrial SSU rRNA from related ciliate *Paramecium tetraurelia* were included. It should be noted that SSU rRNA analysis typically reveals a divergence of the monophyletic RLEs after free-living α-Proteobacteria, but prior to Rickettsiaceae and mitochondria. Moreover, branching order did not depend upon a species sampling (not shown). It is clear (Fig. 1B) that very long branches leading to ciliates push RLEs back to

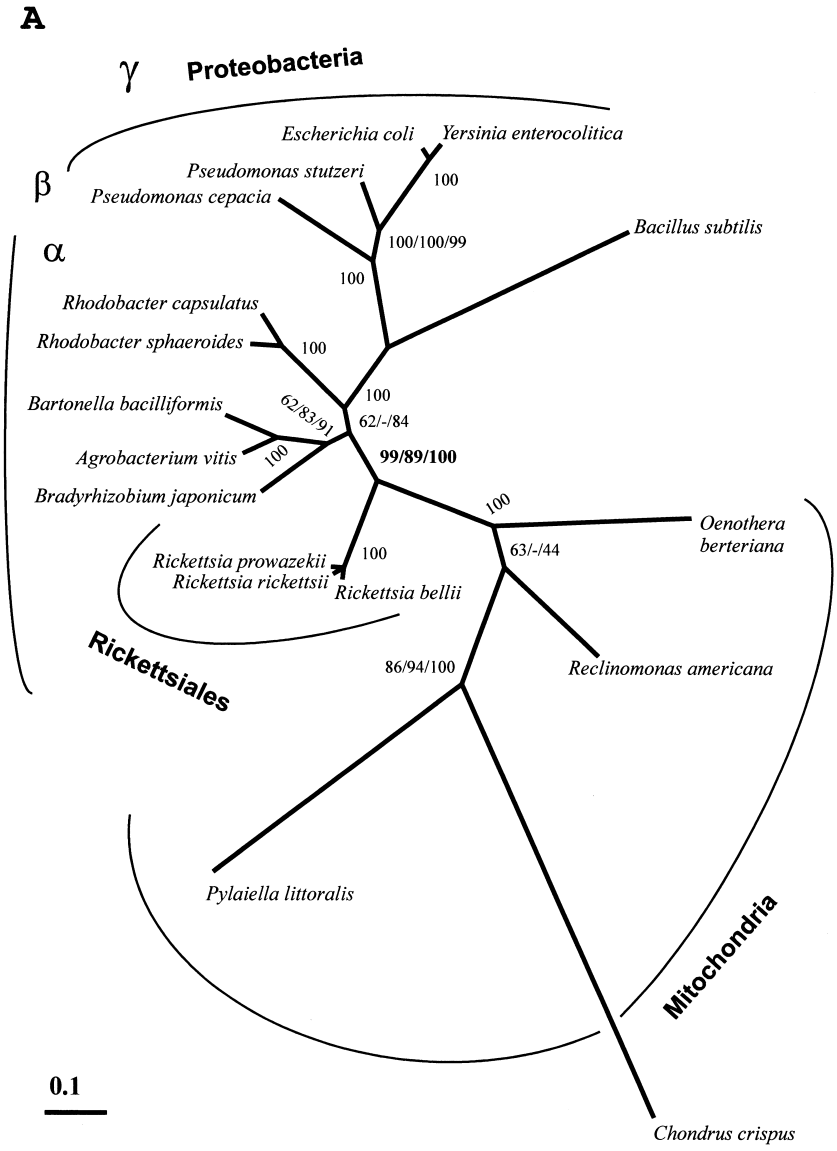

Fig. 1. Phylogenetic relationship among mitochondria and eubacteria inferred from analysis of LSU (A) and SSU (B) rRNA sequences. Bootstrap values (BV) shown in % from left to right (A) or from top to bottom (B) were obtained by ML, DM-based (LogDet program) and MP methods, respectively, as implemented in PAUP 4.0* [42]. These are replaced by dashes, if the topology of the respective tree differs from the one of ML tree. Where only a single BV is shown, support was 100% in all analysis. In ML analysis, the HKY85 model of nucleotide substitutions and gamma distribution of rate variation among sites were used. MP analysis involved the Kimura two-parameter model. Scale bar denotes mean number of substitutions per site for ML tree. In the case of LSU rRNA, both ML-based Kishino–Hasegawa test (PAUP) and MP-based Templeton test (PHYLIP 3.5 [43]) revealed the alternative tree topology, with Rickettsiales transferred to the free-living α-Proteobacteria, to be significantly worse. Mitochondrial sequences were from angiosperms (*O. berteriana* and *Arabidopsis thaliana*), liverwort (*M. polymorpha*), red alga (*C. crispus*), brown alga (*P. littoralis*), ciliates (*P. tetraurelia* and *N. ovalis*), and jakobid (*R. americana*). HGS stands for hydrogenosome. The sequences were obtained from GenBank. Dendrograms were drawn by using the TreeView program.

the free-living relatives. Phylogenetic placement of protists is not, however, compromised by long branch attraction artefact assuming that the branch leading to *R. americana* is the least one.

In contrast to SSU rRNA, intrinsic properties of Cpn60 make it a 'smooth chronometer' perhaps the most appropriate for phylogenetic studies [9,11,15,16]. Fig. 2A shows the results of Cpn60-based analysis performed with ML, distance matrix (DM) and maximum parsimony (MP) methods on a taxonomically 'equilibrated' dataset. These results are in full agreement with former ones [16] and reveal, in particular, divergence of *H. obtusa* before Rickettsiaceae and mitochondria sister groups. Importantly, an affiliation of *T. vaginalis* hydrogenosomal Cpn60 to mitochondrial homologs [15] was firmly corroborated. Because no Cpn60 sequences from other RLEs are available to date, this encouraged me to carry out the analyses of gene sequences using species-reduced input data. As for protein sequences, the species sampling was extensively used. Again, *H. obtusa* was shown to diverge prior to monophyletic cluster of Rickettsiaceae and mitochondria (Fig. 2B).

In summary, both SSU rRNA and Cpn60 analysis revealed that the family Rickettsiaceae and mitochondria had a CA exclusive of the RLEs.

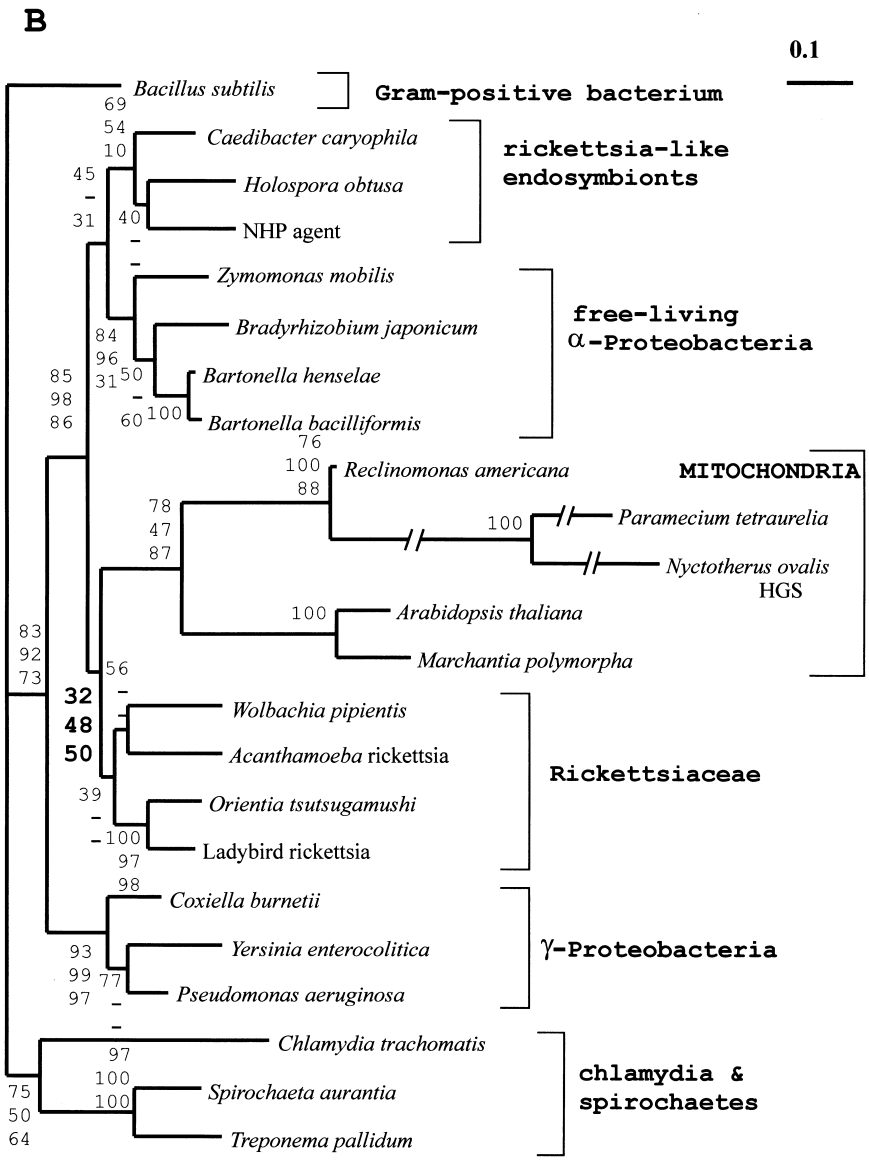

Fig. 1 (*continued*).

4.2. *Rickettsia-like symbiont and common origin of Rickettsiaceae and mitochondria*

Above phylogenetic data strongly suggest that the members of family Rickettsiaceae and mitochondria had a common evolutionary history. The genus *Rickettsia* and mitochondria are closely related groups of descent ([16] and Fig. 2) and as such share some traits derived from CA. In particular, both *R. prowazekii* and aerobic mitochondria possess highly homologous enzymes of TCA and respiration, but lack other energy pathways and most enzymes functioning in small molecule biosynthesis [13]. Accordingly, RLEs are the nearest living relatives of an extinct last CA of Rickettsiaceae and mitochondria (e.g. Fig. 2A) and may therefore, at least in part, reflect an ancestral state. A sequencing of the *H. obtusa* genome has recently been announced [8]. Many important questions are addressed by this study. These deal, first of all, with energy metabolism and biosynthetic pathways. Whether or not the RLEs possess AAC deserves special attention (see below). Five AAC paralogs of *R. prowazekii* have been argued to share common ancestry with two paralogs in *Chlamydia trachomatis*, but not with AAC of mitochondria [13,46]. One may speculate that the gene for bacterial-type AAC appeared first either in *Rickettsia*-like mitochondrial ancestor or in Chlamydiae, and laterally transferred from one taxon to another. An existence of natural rickettsial and chlamydial endosymbionts in *A. castellanii* [28] reinforces this idea suggesting that both groups of microorganisms could in past parasitize the same hosts. It is thought that revelation of the gene(s) encoding bacterial-type AAC in the *H. obtusa* genome would strongly support the classic endosymbiont theory.

5. Conclusions

The endosymbiont theory, in its traditional formulation, posits that mutual advantage of symbiosis was a transfer of respiration-derived ATP from symbiont to anaerobic host in exchange for useful metabolites and physical protection. However, mitochondrial progenitor is often considered as a sort of polypotent α-Proteobacterium resembling its free-living cousins [8]. An overemphasis of this concept entails assumptions

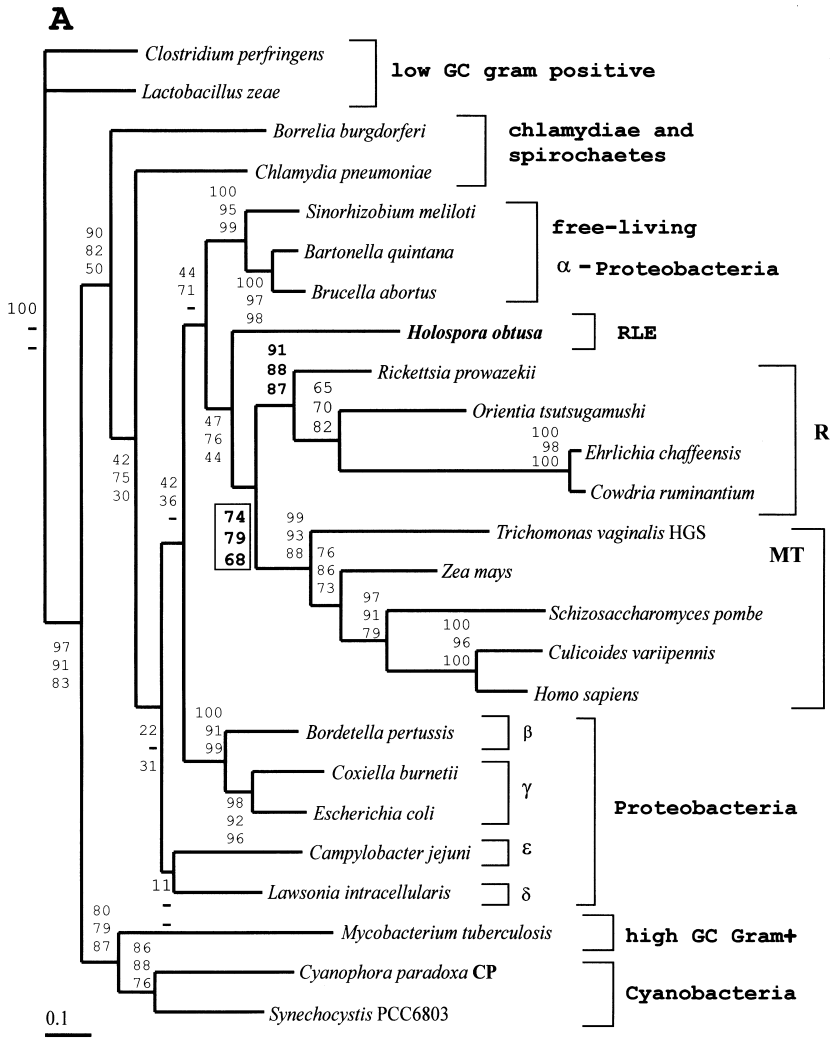

Fig. 2. Phylogenetic relationships of Cpn60 homologs involving protein (A) and gene (B) sequences. In both cases, ML majority rule consensus trees are shown. (A) A dataset contained 504 aligned positions. BV for branching order of ML tree was inferred using ProtML in MOLPHY 2.2 with the JTT-f substitution model [44] and resampling estimated log-likelihood method [10]. DM analysis (FITCH) was carried out with PHYLIP 3.6 package using the Dayhoff model and Jin-Nei correction for among site variation in rates with gamma shape parameter $\alpha = 0.94$ estimated in PUZZLE 4.0 [45]. Unweighted MP analysis was performed by 50 rounds of random stepwise addition heuristic searches with tree bisection-reconnection branch swapping under the minimum evolution criterion (PAUP). (B) The tree was constructed by PUZZLE using the HKY85 model and one invariant-site rate+six variable-site rates. Only the first two codon positions were involved in analysis. Similar trees were obtained using all three methods as installed in PAUP, except that *H. obtusa* always diverged immediately before Rickettsiaceae and organelles while *Rickettsia* and *Orientia* formed a single group. Abbreviations are: MT, mitochondria; R, Rickettsiaceae; RLE, *Rickettsia*-like endosymbiont; CP, chloroplast (cyanella). For other detail see legend to Fig. 1.

that (1) Rickettsiae and mitochondria experienced independent (convergent) reductive evolution; (2) not ATP was initially a mutual benefit of symbiosis, because free-living bacteria lack AAC. Instead, both syntrophy [4,6] and ox-tox hypotheses [7] posit that aerobic respiration served from the start an oxygen-scavenging function.

Present phylogenetic data permit a revitalization of the classic endosymbiont theory. They give rise to an assumption that the common origin of Rickettsiaceae and mitochondria has been predisposed by the long-term endosymbiotic relationship of an intracellular α-Proteobacterium with a primitively amitochondriate host. The latter is usually considered as a full-fledged eukaryote, descended from archaebacterium, which has engulfed mitochondrial symbiont by endocytosis [3]. One may instead suggest that the host has been actually a prokaryote [5], but a chimera created by fusion between archaebacterium and eubacterium, as most vigorously advocated by Gupta [9]. *Rickettsia*-like bacterium could penetrate such a host cell due to membrane-associated phospholipase activity, with plasma membrane being subsequently darned before cytoplasm leakage. Thriving in very rich and safe medium, host cytosol, endosymbiont has invented a variety of carrier proteins and then dispensed with a lot of genes specifying now redundant metabolic pathways such as glycolysis, fermentation, and biosynthesis of small molecules. These are assumed to have been inherited by a pro-eukaryote mostly from eubacterial fusion partner and still present in the host cytoplasm. Being capable of both aerobic and anaerobic respiration, endosymbiotic bacterium produced ATP more efficiently than its host did. An acquisition of AAC is crucial to an establishment of symbiosis. By means of AAC the endosymbiont exported ATP to cytosol, thus allowing the host to better survive. In-

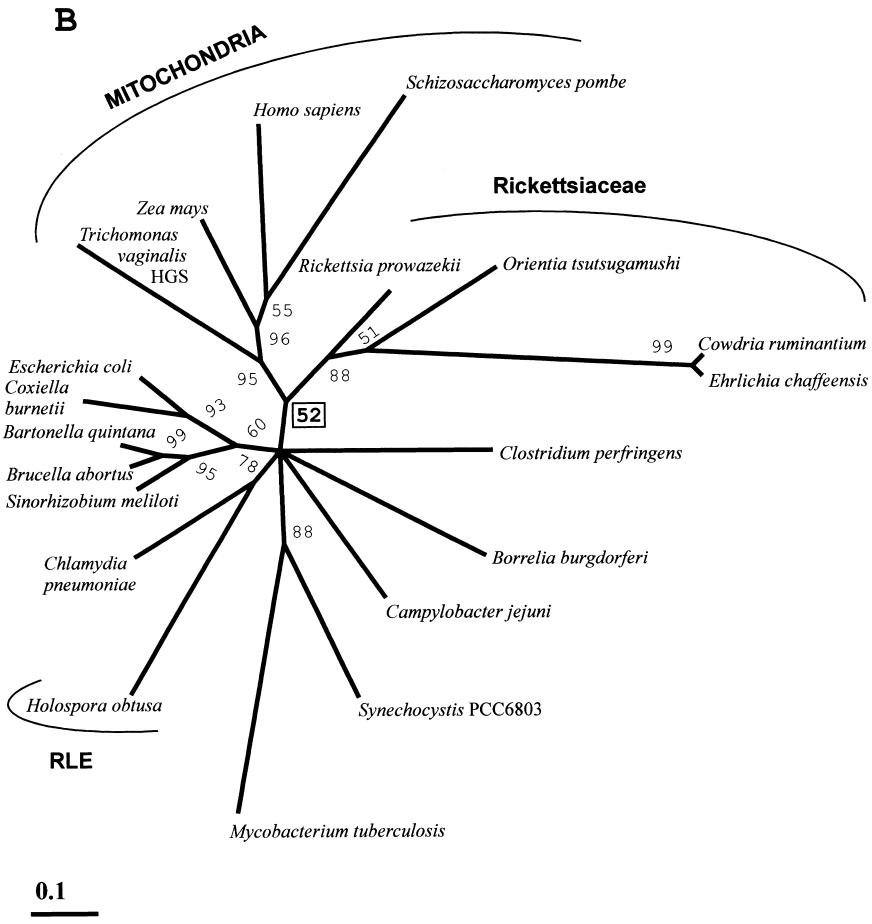

Fig. 2 (*continued*).

deed, despite an apparent absence of homology between bacterial and mitochondrial proteins [13,46], they are functionally similar – both exchange ATP and ADP in an obligate, simultaneous manner [46–48]. A net result of transfer depends, therefore, on the difference between ATP/ADP ratio in different compartments. Thus, the endosymbiotic relationship would be mutualism rather than parasitism. Transfer of some indispensable genes from symbiont to host genome could further stabilize the mutualistic relationship, yet allowing the host to take a partial control over invader. These might have been, first of all, the genes encoding outer membrane proteins with unassisted or loosely assisted incorporation into membrane. It is worth noting in this regard the absence in *R. prowazekii* genome of the gene encoding oxygen sensor TspO. This gene for outer membrane protein, whose mitochondrial homolog encodes peripheral benzodiazepine receptor and resides in nuclear genomes, has been found in the free-living α-Proteobacteria such as *Rhodobacter sphaeroides*, *Paracoccus denitrificans* [49], and *Sinorhizobium meliloti* (unfinished genome).

One may suggest that *Rickettsia* and mitochondria diverged from each other just at this stage. Thus, a molecular basis of obligate rickettsial parasitism may be an import of the proteins encoded by the transferred genes. Carrier proteins [13] are assumed to have been preserved in Rickettsiae from CA. *R. prowazekii* is known to use AAC for ATP import at the onset of infection. However, an expression of the genes for AAC is downregulated, when bacterium produces ATP via respiration in an amount exceeding its level in the cytoplasm of a weakened host [46].

A specific mode of reductive evolution has been initiated in mitochondrial lineage, when a sort of *Rickettsia*-like bacterium described above has lost an ability to escape from a pro-eukaryotic cell. Refinement (or reinvention) of protein import machinery has made it possible the successful transfer to the host genome of the genes specifying biogenesis and energy pathways (TCA and both aerobic and anaerobic respiration). Latter ones, being a true evolutionary novelty, became integrated with the host metabolic network. It is further suggested that AAC could not be targeted back into the emerging organelle after gene transfer to the host genome because it is a highly hydrophobic monomeric protein with 12 transmembrane domains (TMDs) [46]. Notably, Cox1 also contains 12 TMDs [50] and may therefore be incompatible with import into mitochondria (see above). On the contrary, mitochondrial AAC is known to be a dimer with six TMDs in each subunit [47]. It is a member of the paralogous mitochondrial carrier family [48], which is thought to have replaced rickettsial-like carrier proteins in the course of endosymbiont domestication.

Acknowledgements: Thanks are due to Olga K. Mamayeva for technical assistance. I am especially grateful to the staff of the supercomputer center (Moscow), who permitted me to use powerful computers for phylogenetic studies.

References

[1] Margulis, L. (1996) Proc. Natl. Acad. Sci. USA 93, 1071–1076.
[2] Gray, M.W. (1998) Curr. Opin. Genet. Dev. 9, 678–687.
[3] Doolittle, W.F. (1998) Nature 392, 15–16.
[4] Martin, W. and Müller, M. (1998) Nature 392, 37–41.
[5] Vellai, T., Takacs, K. and Vida, G. (1998) J. Mol. Evol. 46, 499–507.
[6] Lopez-Garcia, P. and Moreira, D. (1999) Trends Biochem. Sci. 24, 88–93.
[7] Kurland, C.G. and Andersson, S.G. (2000) Microbiol. Mol. Biol. Rev. 64, 786–820.
[8] Lang, B.F., Gray, M.W. and Burger, G. (1999) Annu. Rev. Genet. 33, 351–397.
[9] Gupta, R.S. (1998) Microbiol. Mol. Biol. Rev. 62, 1435–1491.
[10] Horner, D.S., Hirt, R.P. and Embley, T.M. (1999) Mol. Biol. Evol. 16, 1280–1291.
[11] Viale, A.M. and Arakaki, A.K. (1994) FEBS Lett. 341, 146–151.
[12] Olsen, G.J., Woese, C.R. and Overbeek, R. (1994) J. Bacteriol. 176, 1–6.
[13] Andersson, S.G.E., Zomorodipour, A., Andersson, J.O., Sicheritz-Ponten, T., Alsmark, U.C.M., Podowski, R.M., Näslund, A.K., Eriksson, A.-S., Winkler, H.H. and Kurland, C.G. (1998) Nature 396, 133–140.
[14] Sicheritz-Ponten, T., Kurland, C.G. and Andersson, S.G.E. (1998) Biochim. Biophys. Acta 1365, 545–551.
[15] Roger, A.J. (1999) Am. Nat. 154, S146–S163.
[16] Emelyanov, V.V. and Sinitsyn, B.V. (1999) in: Rickettsiae and Rickettsial Diseases at the Turn of the Third Millennium (Raoult, D. and Brouqui, P., Eds.), pp. 31–37, Elsevier, Paris.
[17] Kobayashi, M., Matsuo, Y., Takimoto, A., Suzuki, S., Maruo, F. and Shoun, H. (1996) J. Biol. Chem. 271, 16263–16267.
[18] Tielens, A.G.M. and van Hellemond, J.J. (1998) Biochim. Biophys. Acta 1365, 71–78.
[19] Cavalier-Smith, T. (1998) Biol. Rev. 73, 203–266.
[20] Embley, T.M., Horner, D.S. and Hirt, R.P. (1997) Trends Ecol. Evol. 12, 437–441.
[21] Amann, R.I., Ludwig, W. and Schleifer, K.-H. (1995) Microbiol. Rev. 59, 143–169.
[22] Brenner, D.J., O'Connor, S.P., Winkler, H.H. and Steigerwalt, A.G. (1993) Int. J. Syst. Bacteriol. 43, 777–786.
[23] Hackstadt, T. (1996) Infect. Agents Dis. 5, 127–143.
[24] Rikihisa, Y. (1999) in: Rickettsiae and Rickettsial Diseases at the Turn of the Third Millennium (Raoult, D. and Brouqui, P., Eds.), pp. 393–405, Elsevier, Paris.
[25] Tamura, A., Ohashi, N., Urakami, H. and Miyamura, S. (1995) Int. J. Syst. Bacteriol. 45, 589–591.
[26] Chen, D., Campbell, B.C. and Purcell, A.H. (1996) Curr. Microbiol. 33, 123–128.
[27] Davis, M.J., Ying, Z., Brunner, B.R., Pantoja, A. and Ferwerda, F.H. (1998) Curr. Microbiol. 36, 80–84.
[28] Fritsche, T.R., Horn, M., Seyedirashti, S., Gautom, R.K., Schleifer, K.-H. and Wagner, M. (1999) Appl. Environ. Microbiol. 65, 206–212.
[29] Stouthamer, R., Breeuwer, J.A.J., Luck, R.F. and Werren, J.H. (1993) Nature 361, 66–68.
[30] Loy, J.K., Dewhirst, F.E., Weber, W., Frelier, P.F., Garbar, T.L., Tasca, S.I. and Templeton, J.W. (1996) Appl. Environ. Microbiol. 62, 3439–3445.
[31] Saraste, M. (1999) Science 283, 1488–1493.
[32] Akhmanova, A., Voncken, F., van Alen, T., van Hoek, A., Boxma, B., Vogels, G., Veenhuis, M. and Hackstein, J.H.P. (1998) Nature 396, 527–528.
[33] Rotte, C., Henze, K., Müller, M. and Martin, W. (2000) Curr. Opin. Microbiol. 3, 481–486.
[34] Müller, M. (1988) Annu. Rev. Microbiol. 42, 465–488.
[35] Dyall, S.D., Koehler, C.M., Delgadillo-Correa, M.G., Bradley, P.J., Plümper, E., Leuenberger, D., Turck, C.W. and Jonson, P.J. (2000) Mol. Cell Biol. 20, 2488–2497.
[36] Neupert, W. (1997) Annu. Rev. Biochem. 66, 863–917.
[37] Scotti, P.A., Urbanus, M.L., Brunner, J., de Gier, J.-W., von Heijne, G., van der Does, C., Driessen, A.J.M., Oudega, B. and Luirink, J. (2000) EMBO J. 19, 542–549.
[38] Hausler, T., Stierhof, Y.D., Blattner, J. and Clayton, C. (1997) Eur. J. Cell Biol. 73, 240–251.
[39] Adams, K.L., Song, K., Roessler, P.G., Nugent, J.M., Doyle, J.L., Doyle, J.J. and Palmer, J.D. (1999) Proc. Natl. Acad. Sci. USA 96, 13863–13868.
[40] Claros, M.G., Perea, J., Shu, Y., Samatey, F.A., Popot, J.L. and Jacq, C. (1995) Eur. J. Biochem. 228, 762–771.
[41] van Hoek, A.H.A.M., Akhmanova, A.S., Huynen, M.A. and Hackstein, J.H.P. (2000) Mol. Biol. Evol. 17, 202–206.
[42] Swofford, D.L. (1998) PAUP*, Phylogenetic Analysis Using Parsimony (* and other methods), version 4.0, Sinauer, Sunderland, MA.
[43] Felsenstein, J. (1999) PHYLIP, Phylogeny Inference Package, version 3.6, University of Washington, Seattle, WA.
[44] Adachi, J. and Hasegawa, M. (1996) Comput. Sci. Monogr. 28, Inst. Stat. Math., Tokyo.
[45] Strimmer, K. and von Haeseler, A. (1996) Mol. Biol. Evol. 13, 964–969.
[46] Winkler, H.H. and Neuhaus, H.E. (1999) Trends Biochem. Sci. 24, 64–68.
[47] Saraste, M. and Walker, J.E. (1982) FEBS Lett. 144, 250–254.
[48] Nelson, D.R., Felix, C.M. and Swanson, J.M. (1998) J. Mol. Biol. 277, 285–308.
[49] Yeliseev, A.A., Krueger, K.E. and Kaplan, S. (1997) Proc. Natl. Acad. Sci. USA 94, 5101–5106.
[50] Castresana, J., Lübben, M., Saraste, M. and Higgins, D.G. (1994) EMBO J. 13, 2516–2525.