# Origins of hydrogenosomes and mitochondria

Commentary

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Complete genome sequences for many oxygen-respiring mitochondria, as well as for some bacteria, leave no doubt that mitochondria are descendants of α-proteobacteria, a finding for which the endosymbiont hypothesis can easily account. Yet a wealth of data indicate that mitochondria and hydrogenosomes – the ATP-producing organelles of many anaerobic protists – share a common ancestry, a finding that traditional formulations of the endosymbiont hypothesis less readily accommodates. Available evidence suggests that a more in-depth understanding of the origins of eukaryotes and their organelles will hinge upon data from the genomes of protists that synthesize ATP without the need for oxygen.

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**Abbreviations**

| | |
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| **FRD** | fumarate reductase |
| **PDH** | pyruvate dehydrogenase |
| **PFO** | pyruvate:ferredoxin oxidoreductase |
| **SDH** | succinate dehydrogenase |

## Introduction

The origins of eukaryotic cells and their characteristic organelles, the mitochondria, are currently among the more hotly debated issues in evolutionary cell biology. In a recent review of the topic [1••], it was surmized that molecular phylogenetic data have "confirmed the simplest version of the endosymbiosis hypothesis", which addresses the origin of aerobic ATP-producing pathways in mitochondria. Furthermore, it was concluded [1••] that the hydrogen hypothesis for the origins of mitochondria, which addresses the origins of anaerobic ATP-producing pathways in hydrogenosomes, does not receive support from molecular data. The hydrogen hypothesis [2••] posits *inter alia* that mitochondria and hydrogenosomes share a common ancestry — a view that is not universally accepted but that is supported by a considerable amount of evidence. This issue invites discussion from an alternative perspective. The purpose of this paper is to point to the diversity of anaerobic ATP synthesis in mitochondria, to briefly recapitulate data indicating a common ancestry of mitochondria and hydrogenosomes, and to raise the question of its evolutionary significance. In principle, the anaerobic biochemistry in these organelles could be the result of widespread lateral gene transfers, hence fortuitous curiosities of evolution. Alternatively, it might be a commonly inherited relic from the earliest era of eukaryotic metabolism, hence a source of insight into biochemical history. These are specific biological questions that can be posed to microbial genome data.

## Hydrogenosomes and mitochondria: biochemical diversity

The simplest version of the endosymbiont hypothesis [1••] accounts for the origin of aerobic mitochondria only — not for the origin of anaerobic mitochondria or hydrogenosomes. In fact, almost without exception, *all* endosymbiotic models for the origin of mitochondria focus on the derivation of a narrow and specific subset of mitochondrial diversity — namely typical, textbook-like, aerobic mitochondria such as those found in cells of the human liver.

Such organelles utilize pyruvate dehydrogenase (PDH) for oxidative decarboxylation, a citric acid cycle to regenerate CoASH for PDH and to produce NADH that is fed into the ATP-producing respiratory chain with $O_2$ serving as the terminal acceptor [1••,2••]. The same biochemistry is found in the obligate aerobe *Rickettsia prowazekii*, the first α-proteobacterium for which a genome sequence is available [3••]. But *Rickettsia* is a highly reduced and specialized α-proteobacterium [4,5,6••] and many free-living α-proteobacteria possess a greater spectrum of biochemical diversity than *Rickettsia*. Similarly, liver-type mitochondria are highly specialized organelles [7•]. Among eukaryotes that inhabit anaerobic environments and among those that have anaerobic stages in their life cycle, there is a wealth of biochemical diversity in mitochondrial energy metabolism that classical formulations of the endosymbiont hypothesis neither account for nor address, arguably because they are designed to explain the origin of an oxygen-consuming organelle.

Specific examples of such anaerobic mitochondria include those of the fungus *Fusarium oxysporum* that perform nitrate-respiration under oxygen-limiting conditions [8,9], and the anaerobic mitochondria of some ciliates, for which nitrate respiration has also been reported [10]. Further examples include fumarate respiration as found in the mitochondria of plathelminthes [11•] and the succinate-producing mitochondria of some trypanosomes [12•]. There are also the facultatively anaerobic mitochondria of the nematode *Ascaris suum*, in which complex II of the respiratory chain functions as succinate dehydrogenase (SDH) during aerobic respiration in the larval stage and as fumarate reductase (FRD) during (anaerobic) fumarate respiration in the adult stage, changes that are accompanied by the expression of proteins both common to and

specific for SDH and FRD activities [13]. Still further examples include the mitochondria of the anaerobic ciliate *Nyctotherus* that perform hydrogen-producing fermentations [14•], and that constitute a previously missing link between mitochondria and hydrogenosomes [15•].

The diversity of pathways for ATP synthesis from pyruvate among anaerobic mitochondria exceeds that found either in typical mitochondria or in the strict aerobe *Rickettsia*. However, it does not exceed the diversity found in typical, facultatively anaerobic α-proteobacteria such as *Paracoccus denitrificans* [16], *Rhodobacter* species or any number of species from this biochemically diverse group [17]. Moreover, such facultative anaerobic bacteria can typically perform $H_2$-producing fermentations just like hydrogenosomes, which are the double-membrane bounded, $H_2$-producing organelles of ATP synthesis that are found in several groups of anaerobic protists.

Hydrogenosomes are known to occur among the trichomonads [18], the ciliates [19], the heteroloboseans [20] and the chytridiomycete fungi [21,22]. Typical hydrogenosomes use pyruvate:ferredoxin oxidoreductase (PFO) for oxidative decarboxylation instead of PDH. Rather than a citric acid cycle, they possess a two-enzyme system consisting of succinate:acetate CoA transferase and succinyl-CoA synthase to regenerate CoASH, thereby synthesizing one mole of ATP per mole of pyruvate. Electrons generated by PFO are transferred to protons as the terminal acceptor via an [Fe] hydrogenase, producing $H_2$ as the final reduced end product [18]. Importantly, just as among mitochondria, there are lineage-specific variations upon this basic biochemical theme [18,23,24,25••,26••].

## Hydrogenosomes and mitochondria: common ancestry

A wealth of evidence indicates that hydrogenosomes are anaerobic forms of mitochondria — that is hydrogenosomes and mitochondria share a common ancestry from a single progenitor organelle. The nature of this evidence is several-fold (reviewed in [23,24,25••,26••,27•]). Like mitochondria, hydrogenosomes of trichomonads [28] and chytridiomycetes [29] are surrounded by two membranes, whereby those in some ciliates even possess distinctively cristae-like structures [30]. Like mitochondria, they are organelles of pyruvate oxidation and ATP production [31], as well as $Ca^{2+}$ storage [22,32]. Furthermore, some have been shown to develop a membrane potential [33]. The mechanism of division (formation of central septum) is similar in mitochondria and hydrogenosomes [34], although no hydrogenosomes and only one group of mitochondria [35•] have been found to possess the FtsZ protein typical of prokaryotic cell division. Like mitochondria, hydrogenosomes import proteins with the help of transit peptides [36] that, although shorter than typical mitochondrial transit peptides, are recognized by the mitochondrial protein import apparatus of trypanosomes [37] and fungi [38] (reviewed in [39•]). Hydrogenosomes import several proteins that are otherwise specific to mitochondria and that branch with mitochondrial homologues in phylogenetic analyses, such as Hsp70, Hsp60, and Hsp10 (reviewed in [24,25••,26••,27•,39•,40••]). Trichomonad hydrogenosomes possess many proteins common to mitochondria, including the α- and β-subunits of succinyl-CoA synthase [40••], an enzyme that in hydrogenosomes is involved in the regeneration of CoASH from acetyl-CoA, analogous to its function in the citric acid cycle of mitochondria. Importantly, they also possess a homologue of the mitochondrial ADP-ATP translocase [41•].

Although most hydrogenosomes do not contain a genome [18,42], those of the ciliate *Nyctotherus ovalis* do [14•]. The 16S rRNA sequence from this DNA indicates a ciliate mitochondrial ancestry of the hydrogenosomal genome [43]. Among the ciliates, mitochondrion- and hydrogenosome-bearing forms are highly interleaved in molecular phylogenies, indicating common ancestry of the organelles [19]. Among the fungi, hydrogenosome-bearing forms also occur interleaved with mitochondrion-bearing forms. For example, the hydrogenosomes of the chytridiomycete *Neocallimastix* possess PFO activity and produce a mixture of hydrogen and formate, depending upon growth conditions [44]. Another example is the chytridiomycete *Piromyces*, from which a number of mitochondrially related genes have been identified via database searches with expressed sequence tags (ESTs) [45].

## How to account for the origin of anaerobic organelles?

What biological models do we have that can account for the data linking hydrogenosomes with mitochondria and for the origin of ATP-producing pathways in anaerobic mitochondria and hydrogenosomes? Clearly, since its resurrection [46] from earlier versions, (e.g. [47]) various formulations of the endosymbiont hypothesis over the years have focused on the origin of ATP synthesis in aerobic mitochondria [16,48–51,52•]. During this same period, sequences of many mitochondrial genomes have become known, all of which encode one or the other component of the mitochondrial electron transport chain [6••,53•] (a noteworthy finding that deserves explanation in its own right [54,55•,56•]).

Yet at the same time as molecular sequence data from mitochondrial and α-proteobacterial genomes were accumulating, a great deal of progress was being made in the biochemical and cytological study of anaerobic mitochondria and hydrogenosomes [18,24,26••,57]. However, various formulations and reformulations of the endosymbiont hypothesis did not incorporate findings from these anaerobic organelles in a manner in which they could be explained. Accordingly, hydrogenosomes remained largely outside the scope of mainstream endosymbiotic theory until their evolutionary affinity with mitochondria became virtually undeniable [23,24,25••,26••,27•]. Even then, in a theory that was designed to explain the origin of an oxygen-respiring organelle, there was no room for anaerobic biochemistry.

Novel and intriguing symbiotic models are emerging to account for the differences between prokaryotes and eukaryotes at the level of cellular organization and genome complexity [58•,59,60•,61]. These have distinctive virtues but do not directly account for the diversity and compartmentation of ATP-producing pathways observed among contemporary anaerobic protists.

Today, there still are basically two ways to explain the origin of anaerobic biochemistry in hydrogenosomes. Under one alternative, the ancestral mitochondrion is viewed as an oxygen-respiring organelle in adherence to traditional formulations of the endosymbiont hypothesis, and the genes for the enzymes specific to ATP synthesis in anaerobic mitochondria and hydrogenosomes are viewed as acquisitions involving independent lateral gene transfer events in different eukaryotic lineages [1••]. Under a different alternative, the common ancestor of mitochondria and hydrogenosomes is viewed as a facultatively anaerobic α-proteobacterium that was able to satisfy its ATP needs with and without the help of oxygen, whereby the imprint of this facultatively anaerobic past is preserved in the spectrum of organelle diversity that is observed among protists today [2••].

Although based upon data from the study of only a handful of anaerobic protists [18,26••], the hydrogen hypothesis [2••] generates a number of testable predictions concerning those anaerobic protists that have not yet been studied in molecular or biochemical detail — particularly amitochondriate ones. It predicts all mitochondrion-lacking, nucleated cells to be secondarily amitochondriate, that is to have possessed a mitochondrial/hydrogenosomal symbiont in their evolutionary past but to have subsequently lost the organelle through reduction. This provides a reasonably simple criterion by which it can be falsified in gene and genome comparisons. It also predicts that eukaryotic nuclear genes for proteins involved in energy metabolism in hydrogenosomes should share a single eubacterial origin. A recent phylogeny of the four available eukaryotic PFO sequences indicates that these genes do seem to stem from a single eubacterial source, although they cannot currently be traced to an α-proteobacterial donor [62•].

Notably, PFO in the cytosol of *Entamoeba histolytica* shares a common ancestry with its homologue from hydrogenosomes [62•]. This is consistent with the recent discovery in *Entamoeba* of a surprising relictual mitochondrion, termed the mitosome [63••] (or crypton [64••]), an organelle that has apparently lost its function in energy metabolism, suggesting that it represents an intermediate stage in the organelle reduction process [63••].

## Conclusions

Twenty years ago, biologists were debating whether respiration in mitochondria is an inheritance from purple non-sulfur bacteria [49]. That debate is over, thanks in no small part to the sequencing of the genome of *Rickettsia prowazekii* [1••,3••]. But the time has come to address the origin of biochemistry of hydrogenosomes and anaerobic mitochondria in endosymbiotic models.

Five years ago, biologists began debating whether contemporary oxygen-shunning eukaryotes that lack mitochondria have secondarily lost the organelle, since nuclear genes of mitochondrial origin were found in amitochondriate protists [65]. Such findings have since been extended to many amitochondriate groups and have rightly prompted critical reinspection of our views on how eukaryotes and their characteristic organelles arose [2••,24,26••,52•,66••].

Today, we know that mitochondria descend from α-proteobacteria because comparative genome data permit no other interpretation [3••,6••,66••]. Yet there is still a tendency to presume that the host was an anaerobic, heterotrophic, phagocytotic cell, often envisaged as an organism organized similarly to contemporary amitochondriate eukaryotes that inhabit anaerobic niches [1••]. This view has a very long tradition in endosymbiotic thinking [67], making it all the more important that we critically reinspect its merits in light of newer findings, because the endosymbiont hypothesis has fared much better when it comes to explaining the origins of organelles than it has when it comes to explaining the origin of their host [68].

It may well turn out that the various groups of eukaryotes that today inhabit anaerobic (and hypoxic) environments have acquired the genes necessary to colonize these niches via independent lateral transfers. Or it may turn out that commonly inherited biochemical relics from the anaerobic past have been preserved throughout eukaryotic history. And it is possible that the truth will lie somewhere in between. However, we can be sure that many of the genes that have found their way into eukaryotic chromosomes, by whatever means, will have been used as genetic starting material to give rise to novel functions [69•]. Clearly, the study of eukaryotes that do not depend upon oxygen will provide the incisive clues.

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56. • Berg OG, Kurland CG: **Why mitochondrial genes are most often found in nuclei.** *Mol Biol Evol* 2000, **17**:951-961.
Mitochondria have highly reduced genomes – much of what was once a complete genome of a free-living α-proteobacterium has ended up in nuclear chromosomes. This paper provides a straightforward population genetic model to explain why that should be the case.

57. Johnson PJ, Lahti CJ, Bradley PJ: **Biogenesis of the hydrogenosome in the anaerobic protist *Trichomonas vaginalis*.** *J Parasitol* 1993, **79**:664-670.

58. • Vellai T, Takács K, Vida G: **A new aspect to the origin and evolution of eukaryotes.** *J Mol Evol* 1998, **46**:499-507.
This paper posits that a mitochondrion-bearing cell arose from a symbiosis of an archaebacterial host with an α-proteobacterial symbiont, focusing on increased genome size among eukaryotes and how oxygen-driven ATP synthesis in mitochochondria conceivably could have augmented that process. In this view, all amitochondriate protists would descend from mitochondriate forebearers, and hydrogenosomes would derive from mitochondria.

59. Vellai T, Vida G: **The origin of eukaryotes: the difference between prokaryotic and eukaryotic cells.** *Proc R Soc Lond B* 1999, **266**:1571-1577

60. • Moreira D, Lopez-Garcia P: **Symbiosis between methanogenic archaea and δ-proteobacteria as the origin of eukaryotes: the syntrophic hypothesis.** *J Mol Evol* 1998, **47**:517-530.
The syntrophic hypothesis posits that a eubacterial host acquired an archaebacterial symbiont, which became the nucleus in the resulting, primitively amitochondriate eukaryote. This cell then acquired an anaerobic, methanotrophic α-proteobacterium as the mitochondrial symbiont.

61. Lopez-Garcia P, Moreira D: **Metabolic symbiosis at the origin of eukaryotes.** *Trends Biochem Sci* 1999, **24**:88-93.

62. • Horner DS, Hirt RP, Embley TM: **A single eubacterial origin of eukaryotic pyruvate: ferredoxin oxidoreductase genes: implications for the evolution of anaerobic eukaryotes.** *Mol Biol Evol* 1999, **16**:1280-1291.
The anaerobic lifestyle has arisen many times independently among eukaryotes, but where do the genes come from that are essential for anaerobic energy metabolism in eukaryotes that lack mitochondria? The phylogeny of pyrovate:ferredoxin oxidoreductase genes bears heavily

upon this issue and the data in this paper indicate a single origin for the eukaryotic enzyme.

63. Tovar J, Fischer A, Clark CG: **The mitosome, a novel organelle**
•• **related to mitochondria in the amitochondrial parasite *Entamoeba histolytica*.** *Mol Microbiol* 1999, **32**:1013-1021.

*Entamoeba* was long believed to lack an organelle related to mitochondria. Here, a homologue of the mitochondrial chaperonin Cpn60 from this protist is shown to possess an amino-terminal transit peptide that directs import into an inconspicuous and previously overlooked organelle in *Entamoeba*. The mitochondrial targeting signal of *Trypanosoma* Hsp70 also targets protein to this organelle, providing strong evidence that it is a reduced mitochondrion.

64. Mai ZM, Ghosh S, Frisardi M, Rosenthal B, Rogers R, Samuelson J:
•• **Hsp60 is targeted to a cryptic mitochondrion-derived organelle ("crypton") in the microaerophilic protozoan parasite *Entamoeba histolytica*.** *Mol Cell Biol* 1999, **19**:2198-2205.

An independent report describing the import of mitchondrial Cpn60 into the novel and unusual reduced mitochondrion of *Entamoeba*.

65. Clark CG, Roger AJ: **Direct evidence for secondary loss of mitochondria in *Entamoeba histolytica*.** *Proc Natl Acad Sci USA* 1995, **92**:6518-6521.

66. Lang BF, Gray MW, Burger G: **Mitochondrial genome evolution and**
•• **the origin of eukaryotes**. *Annu Rev Genet* 1999, **33**:351-397.

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67. Biagini GA, Bernard C: **Primitive anaerobic protozoa: a false concept?** *Microbiology* 2000, **146**:1019-1020.

68. Martin W: **Primitive anaerobic protozoa: the wrong host for mitochondria and hydrogenosomes?** *Microbiology* 2000, **146**:1021-1022.

69. Barton RM, Worman HJ: **Prenylated prelamin A interacts with Narf,**
• **a novel nuclear protein.** *J Biol Chem* 1999, **274**:30008-300018.

Iron only hydrogenases are typical enzymes of hydrogenosomes. This paper reports a gene found in the nuclei of mitochondrion-bearing eukaryotes that is closely related to iron only hydrogenases, but that apparently does not function in energy metabolism. Rather, the protein seems to be associated with lamins of the nuclear envelope.