

Metabolic integration during the evolutionary origin of mitochondria

DENNIS G SEARCY

Biology Department, University of Massachusetts, Amherst MA 01003-9297, USA

ABSTRACT

Although mitochondria provide eukaryotic cells with certain metabolic advantages, in other ways they may be disadvantageous. For example, mitochondria produce reactive oxygen species that damage both nucleocytoplasm and mitochondria, resulting in mutations, diseases, and aging. The relationship of mitochondria to the cytoplasm is best understood in the context of evolutionary history. Although it is clear that mitochondria evolved from symbiotic bacteria, the exact nature of the initial symbiosis is a matter of continuing debate. The exchange of nutrients between host and symbiont may have differed from that between the cytoplasm and mitochondria in modern cells. Speculations about the initial relationships include the following. (1) The pre-mitochondrion may have been an invasive, parasitic bacterium. The host did not benefit. (2) The relationship was a nutritional syntrophy based upon transfer of organic acids from host to symbiont. (3) The relationship was a syntrophy based upon H₂ transfer from symbiont to host, where the host was a methanogen. (4) There was a syntrophy based upon reciprocal exchange of sulfur compounds. The last conjecture receives support from our detection in eukaryotic cells of substantial H₂S-oxidizing activity in mitochondria, and sulfur-reducing activity in the cytoplasm.

Key words: *mitochondria (origin), symbiosis, sulfur, rickettsia, hydrogenosome.*

INTRODUCTION

Mitochondria are a characteristic feature of eukaryotic cells, typically accounting in each cell for > 90% of the O₂ consumed and ATP produced. Mitochondrial dysfunction can cause a variety of diseases, and even healthy mitochondria continuously damage themselves and the surrounding nucleocytoplasm. Thus, the relationship of mitochondria to the rest of the cell is worth examining closely, and best understood from the perspective of evolution.

To show how mitochondria can be important and

interesting, first I describe selected aspects of mitochondria related to human diseases, and then several ideas about the origin of mitochondria.

Aging and Disease

Much of the damage that accumulates with age is caused by Reactive Oxygen Species (ROS), which are O₂ molecules that have not been completely reduced to H₂O. For example, it has been established that up to 4% of the O₂ that each cell consumes is released as O₂⁻ (superoxide)[1], which is an unstable free radical that reacts further to produce a variety of ROS such as OH[·] and H₂O₂[2]. The ROS react with lipids, proteins, and nucleic acids, causing damage that accumulates and is a major source of aging[3]. For example, over 100 types of oxidative damage to DNA have been described, causing mutations that lead to cancer and

* Correspondence: Prof. Dennis G SEARCY
E-mail: dsearcy@bio.umass.edu Fax: (413) 545 3243

Abbreviations and definitions: ROS, reactive oxygen species including O₂⁻, OH[·], and H₂O₂. "Nucleocytoplasm", the nucleus and cytoplasm of a eukaryotic cell, excluding mitochondria, chloroplasts, and symbionts. "Redox", relating to chemical reduction and oxidation reactions.

various of other diseases[4].

Mitochondrial ROS damage not only the nucleocytoplasm, but also the mitochondria themselves[5]. Mitochondrial DNA mutates at 10 times the rate of the nuclear DNA[6]. As the damage accumulates, mitochondria lose function. In a 65 year old human about half of the mitochondrial DNA molecules are damaged[7], with a similar loss in mitochondrial respiratory capacity[8].

Nerve cells are particularly sensitive to a decline in mitochondrial function because they are small cells, but have large energy needs. There is little excess capacity for ATP production, and so any significant decline in mitochondrial function can lead to cell death[9]. Age-related loss of mitochondrial function is believed to contribute to Parkinson's Disease, Alzheimer's Disease, and late-onset Type 2 Diabetes[2].

One example of the type of disease caused by mitochondrial dysfunction is Leber's Hereditary Optic Neuropathy (LHON)[6]. LHON can be caused by any one of nearly 2 dozen different mutations in the mitochondrial DNA. The mutations interfere with ATP production, causing retinal cell death, and blindness by about age 30. An excellent resource for technical information about LHON and other mitochondrial mutations is "Online Mendelian Inheritance in Man" (www.ncbi.nlm.nih.gov/omim/).

Why do cells have mitochondria?

When a question such as that in the heading above is put to a beginning biology student, the student will quickly answer, "The function of mitochondria is to supply the cell with ATP." But bacterial cells also make ATP, and without a separate cytoplasmic compartment. In principle, it is not clear why cells have mitochondria.

Not all aspects of having mitochondria are beneficial to the cell. For example, consider the following. In eukaryotic cells there is redundancy between the genetic apparatus of the mitochondria and the nucleocytoplasm. There are several mitochondria in each cell, and each has several DNA molecules, plus DNA and RNA polymerases, tRNA's, aminoacyl-tRNA synthetases, rRNA's, ribosomal proteins, and more. The mitochondrial functions are evidently identical with those in the nucleocytoplasm; the du-

plication seems unnecessary and wasteful.

The rate of progressive evolution is limited in mitochondria because they do not have a sexual reproductive cycle. The benefit of a sexual cycle is that there is a gene pool shared by many organisms. Instead, mitochondria reproduce clonally. During sexual reproduction of the nucleocytoplasm, mitochondria typically are inherited from only the female parent. Mitochondrial evolutionary change is mostly neutral or reductive.

Since mitochondrial reproduction is independent from that of the nucleocytoplasm, it must be regulated separately. If such regulation fails and mitochondria reproduce too slowly or too rapidly, as sometimes must occur, dire consequences to the cell are obvious.

Problems such as those above could be avoided if the nucleocytoplasm provided its own respiratory functions, and did not use mitochondria. Nonetheless, eukaryotic cells with mitochondria are highly successful. Possible benefits associated with having mitochondria are outlined next below.

One benefit of having mitochondria is metabolic compartmentation. Localizing a metabolic pathway into a specialized compartment provides greater efficiency because, when a compartment excludes some solutes, the enzymes and substrates that remain in the compartment can have higher concentrations, and metabolism can proceed more rapidly. In such a compartment, diffusional distances are shorter. A general organizing principle of eukaryotic cells is compartmentation, and mitochondria are the specific compartments that are specialized for fatty acid oxidation, Krebs Cycle, and oxidative phosphorylation.

A second benefit of compartmentation is protection. For example, it should be advantageous to separate (insofar as possible) the reactions that produce ROS from the cytoplasm and DNA. The nuclear envelope provides further protection for the DNA, and could have been the reason that the nuclear envelope first evolved.

A third benefit of mitochondrial acquisition might have been, in effect, massive gene acquisition. By acquiring the mitochondrial genome the nucleocytoplasm acquired a suite of enzymes that were perhaps entirely absent, or "superior" in some

way to the enzymes initially present. For example, the glucose-metabolizing pathway initially present in the ancestor of the nucleocytoplasm was probably a modified Entner- Douderoff pathway[10]. In modern eukaryotes, it now has been replaced by the Embden-Meyhof pathway, which is of Bacterial origin[11]. One way that the Embden-Meyerhof pathway is "superior" is that it produces 2 ATP per glucose molecule converted to pyruvate, compared to 1 ATP in the Enter-Douderoff pathway.

The Krebs Cycle is more problematic to explain, since both Archaea and Bacteria have Krebs Cycles that are apparently identical[10]. In modern eukaryotic cells there is only one Krebs Cycle, and it is inside the mitochondria, and Bacterial in origin. Hypothetically, when eukaryotic cells switched over to a mitochondrial Krebs Cycle, benefits of compartmentalization were realized, and the cytoplasmic Krebs Cycle was abandoned.

To be provocative, one might suggest that mitochondria are not the best way to organize a cell's energy metabolism. Instead, the modern arrangement might be an "accident of evolution", meaning that it was not the best option but it evolved quickly and precluded other possible alternatives. That is at least partially true for many biological features.

In conclusion, "benefits" and "costs" are not mutually exclusive. All of the considerations described above might have contributed in some way during the origin of mitochondria. To go further into the origin of mitochondria, we now ask, "What were the identities the ancient host and bacterial symbiont?" And, "What nutrients were exchanged between them?" That is considered next below.

Ancestors of nucleocytoplasm and of mitochondria

The nucleocytoplasm shares ancestry with Archaea, as suggested years ago[12]. In most biology textbooks, the "Universal Tree of Life" shows eukaryotes branching off from the bottom of the Archaeal Domain, suggesting that the ancestor of the nucleocytoplasm resembled a primitive Archeon.

The modern organism *Thermoplasma acidophilum* is not a basal Archeon, but nonetheless has several features that make it a good model for the ancestral nucleocytoplasm. (1) It has a histone, which functions to protect the DNA from heat[13]. Simple histone-like proteins occur throughout the

Archaea, but are particularly well developed in the thermophilic species[14-16]. (2) *T. acidophilum* has no cell wall, and instead has a well-developed cytoskeleton[17]. The absence of cell wall is presumably an adaptation for contact respiration upon elemental sulfur. (3) *T. acidophilum* is a facultative aerobe, consistent with the putative aerobic nature of the ancestral nucleocytoplasm. (See later.) (4) There is evidence of a calcium signaling pathway[18]. (5) Protein degradation in *T. acidophilum* uses proteasomes [19]. (6) In *T. acidophilum*, as in all Archaea and Eukaryota, surface proteins are modified by N-glycosylation, meaning that carbohydrate groups are attached to proteins at nitrogen atoms. The synthesis of N-glycoproteins occurs by transfer of a large oligosaccharide all at once from dolichol phosphate to the protein[20]. Nothing like that occurs in Bacteria.

The above features are not found in Bacteria but are universal in eukaryotes, suggesting that they are primitive and should be expected in the ancestor of the nucleocytoplasm.

Regarding the ancestry of mitochondria, there is no doubt that they originated from symbiotic Bacteria[21, 22]. Mitochondria have numerous bacterial features, including the following. (1) New mitochondria arise only by the division of existing mitochondria. Like any living cell, they do not appear de novo. If they are lost or damaged, the host nucleocytoplasm cannot replace them. (2) Mitochondria never separate from nor fuse with any other membrane in the cytoplasm. Mitochondria are always separate entities. (3) Mitochondria have their own DNA and protein synthetic machinery, which are of bacterial type and distinct from that in the nucleocytoplasm. (4) Mitochondrial protein and rRNA sequences are most similar to bacterial sequences. Within the Bacteria evolutionary tree, mitochondria are in the a-Proteobacteria group. (5) Genes for many mitochondrial proteins are located in the eukaryotic nucleus. Evidently that is a result of gene transfer from their original location in ancient mitochondria to the eukaryotic nucleus. Even in the nucleus, the sequences retain their close identity with a-Proteobacterial genes. For example, cytochrome c is encoded in nuclear DNA but has features diagnostic of a-Proteobacteria, and specifically of the Rhodospirillaceae family of purple sulfur bacteria[23].

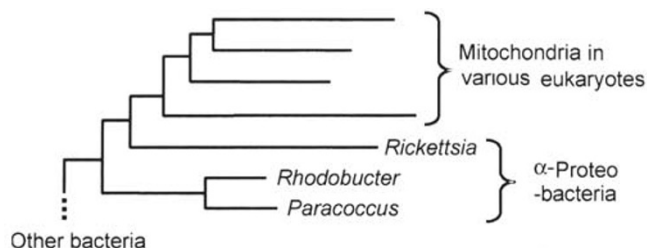


Fig 1. Evolutionary relationships of mitochondria based upon succinic dehydrogenase gene sequences. The diagram shows that *Rickettsia* diverged most recently from mitochondria, but the sequence similarity (= short sum of horizontal branch lengths) is greatest with *Rhodobacter*. Modified from ref[24].

Fig 1 shows the evolutionary relationships of mitochondria obtained from succinic dehydrogenase genetic sequences[24]. The branching sequence indicates that mitochondria diverged most recently from the intracellular parasite *Rickettsia*. The amounts of genetic change are shown by the lengths of the horizontal lines, and indicate that mitochondria are most similar to *Rhodobacter*, a purple sulfur bacterium in the Rhodospirillaceae.

Analyses of rRNA sequences yield similar relationships[25]; mitochondria diverged most recently from rickettsias, but mitochondria are most similar to purple sulfur bacteria. Thus, different conclusions are suggested by different criteria, and the question remains unanswered whether mitochondria evolved from organisms that resembled purple sulfur bacteria or parasitic rickettsias. *Rickettsias* will be discussed in the next section.

In conclusion, what is known is that mitochondria evolved from α-Proteobacteria, and the nucleocytoplasm evolved primarily from a basal Archeon. However, that does not explain why these two organisms initially became associated, nor the nutritional relationship between them. Speculations concerning those questions are considered next.

Mitochondria originated from a pathogenic infection (Fig 2)

According to this conjecture, a bacterium invaded the ancestral nucleocytoplasm, making it sick but not killing it. Over time, the infection became less pathogenic, and eventually evolved into mitochondria.

In parasitology there is the generalization that recently evolved parasites are more pathogenic than those that have been associated with their hosts for long periods of time[26]. The logic is that when parasites do not greatly reduce the evolutionary fitness of their hosts, the hosts and therefore also the parasites can live longer and have greater reproductive success. Mathematical modeling shows that to be particularly true when the parasitic infection is transmitted vertically from mother to daughter, as is the case with mitochondria.

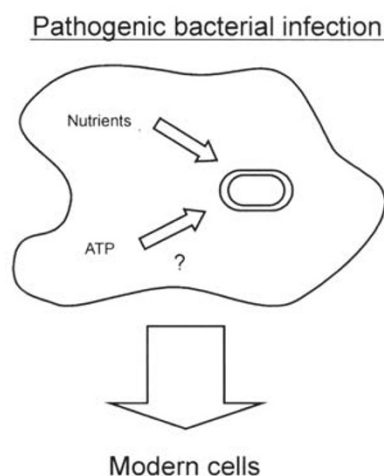


Fig 2. Mitochondrial origin by infection. The ancestor of mitochondria was an intracellular parasite resembling rickettsia, absorbing organic nutrients and possibly ATP from the host cell. The infection was initially harmful, but over time evolved into a beneficial mutualism. For "Modern Cells" see Fig 4, where the logic of the modern arrangement can be understood most easily.

This hypothesis has much to support it. For example, mitochondria are most recently diverged from rickettsias (Fig 1), which are intracellular parasites that cause typhus and other diseases. However, known rickettsias can reproduce only inside animal cells, meaning only inside cells that already have mitochondria. The next paragraph describes more evidence that rickettsias cannot be the ancestors of mitochondria.

The genome of *Rickettsia prowazekii* has been entirely sequenced[24]. There are a large number of pseudogenes in the genome, which are DNA sequences that resemble protein genes but are not actually expressed. The explanation for the pseudogenes is that *R. prowazekii*, as an intracellu-

lar parasite, has many functions provided by its host cell. Since there is no evolutionary selection to maintain these functions in *R. prowazekii*, they accumulate random mutations and become inactivated. Eventually, the non-functional DNA will be removed by genetic deletions, but *R. prowazekii* is still at an early stage in the process. The genome is still relatively large, containing 834 protein genes and 267 kb of non-coding DNA. In contrast, human mitochondrial DNA contains 13 protein genes and 1.5 kb of non-coding DNA, suggesting that mitochondria are ancient and the process of genetic reduction has gone nearly to completion. Thus, mitochondria are older, and cannot be descended from recently evolved rickettsias.

Nonetheless, mitochondria and rickettsia are closely related. (Fig 1) Presumably, a freeliving ancestral bacteria invaded the eukaryotic cytoplasm at least twice-the first time to give origin to mitochondria, and the second time to give origin to rickettsias.

A different type of observation lends further support to the "origin-by-infection" hypothesis. There is a laboratory strain of *Amoeba proteus* that became infected by bacteria[27]. During the initial infection many of the amoebas died, although some survived. Now, after several years, the amoebas have recovered some of their vigor, and the bacteria are still present. Initially, the amoebas could be cured of the infection by antibiotics, but now, when the bacteria are removed the amoebas die, indicating that they have become dependent in some way upon the bacteria. It is not known why or how the amoebas are dependent on the bacteria.

The "origin-by-infection" scenario has much to recommend it. Nevertheless, criticisms can be made as follow. (1) The infected nucleocytoplasm was initially sick, and could not have survived in nature. That is certainly true for rickettsia-like infections, and also in the example of the laboratory amoebas, where even today they are less fit than non-infected cells. (2) One might argue that although the infection reduced the fitness of the host cells, the infection nevertheless became established because the host cells had no way to resist. That is seldom the case in nature, where natural variation causes some cells to be more resistant to infection than others. Also, some cells actually are able to expel mitochondria, such as oc-

curs during the maturation of mammalian red blood cells. Similarly, mitochondria have been expelled during the evolution of certain anaerobic protozoa, such as *Giardia*. (3) If infection decreases the evolutionary fitness of the host, then the host will naturally evolve defenses, including making nutrients less available to the parasite. Thus, in response to a harmful infection, the host evolves in the direction of decreased metabolic integration with the parasite. In contrast, mutually beneficial symbioses will have the opposite effect, as described next below.

Mitochondrial origin from a mutualistic symbiosis

The alternative to pathogenic infection is a symbiosis in which both host and symbiont benefit immediately. The benefit might be some type of protection or might be an exchange of nutrients. For example, in termites the gut symbionts are in a protected environment and receive masticated cellulose. In exchange, they provide soluble nutrients to the termite.

Other symbiotic associations are based entirely on nutrient transfers, and sometimes are called "syntrophies"[28]. For example, there are methanogenic syntrophies in which one species oxidizes organic compounds to H_2 and CO_2 and a second species uses those products to make CH_4 . The first species is inhibited by an accumulation of H_2 , and so it is dependent upon the second organism to remove H_2 . Each species is dependent upon the other, so the relationship is stable.

When a symbiont is beneficial to its host, the host's evolution will be in a direction that facilitates the association. For example, the host may provide nutrients to the symbiont. The direction of evolution will be to increase nutrient exchange and metabolic integration. Thus, mitochondria could evolve more easily from a mutualistic symbiosis than from a parasitic infection.

Among those who agree that mitochondria originated in a mutualistic symbiosis, there is still substantial disagreement about what the transferred nutrients (or other benefits) might have been, as described next.

Ancient mitochondria protected their hosts from oxygen toxicity (Fig 3)

By this conjecture, the ancestor of the nucleocytoplasm was an O_2 -intolerant fermentative cell. It became associated with aerobic bacteria that served to consume any O_2 present, thereby protecting the host cell from O_2 toxicity. In return, the host provided nutrients such as pyruvate to the bacteria. This has been called the "OxTox Model"[29]. As time progressed, the bacterium became intracellular, ATP export was added, and it evolved into modern mitochondria.

One criticism is that it is hard to imagine how, once it was internalized, the symbiont could shield the host cell from O_2 , since O_2 would have to pass through the host's cytoplasm before it could be consumed. It would be more logical to find the O_2 -sensitive cells on the inside, as is the case with O_2 -sensitive methanogens that are harbored in the cytoplasm of certain protozoa[30].

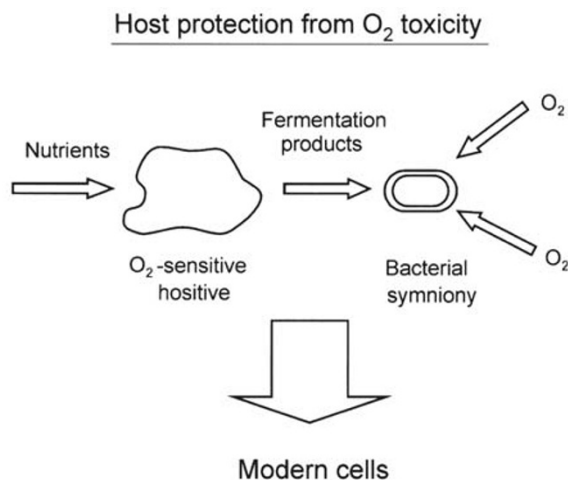


Fig 3. Mitochondrial origin from an association that protected an O_2 -sensitive host cell from O_2 . The bacterial symbiont consumed O_2 , keeping O_2 at a low concentration, and protecting the host cell from O_2 -toxicity. The host cell (ancestor of the nucleocytoplasm) was fermentative, and provided organic nutrients to the bacterial symbiont.

Mitochondrial respiration releases toxic ROS. It is not clear that adding such a respiratory symbiont would be beneficial instead of harmful to the host cell. The harmful consequences of having mitochondria might explain why mitochondria are eliminated from mammalian red blood cells-to protect the hemoglobin from reaction with ROS. Thus, from the standpoint of damage to the host cell, it is not clear that adding a respiratory symbiont protects the host

cell.

Organic acid syntrophy (Fig 4)

This hypothesis was the first to be suggested, and was modeled on the modern relationship of the cytoplasm and mitochondria[31-33]. The host cell fermented organic matter such as glucose to organic acids such as pyruvate and lactate. The acids were transferred to the bacterial symbiont, which oxidized them to CO_2 . The initial function provided by the bacterium was to dispose of the host's acidic waste products.

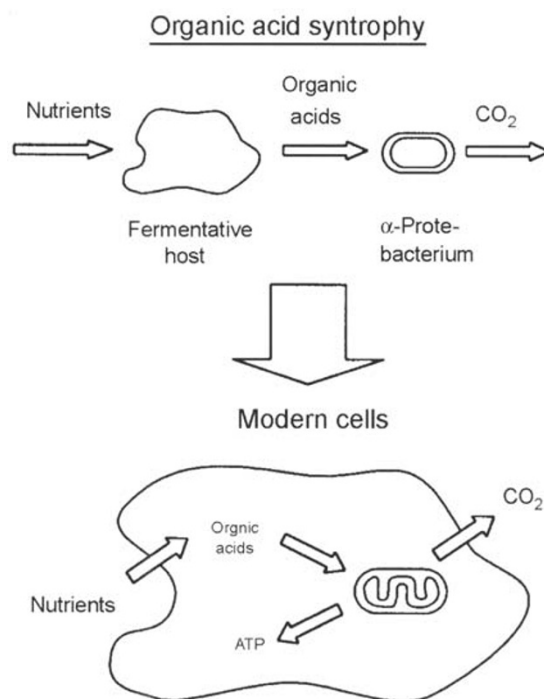


Fig 4. Mitochondrial origin from organic acid syntrophy. The ancestor of the nucleocytoplasm (= host) was anaerobic, fermentative, and consumed nutrients such as carbohydrates. It excreted organic acids such as pyruvate, acetate, succinate, and glutamate, which were oxidized by the bacterial symbiont to CO_2 . The host benefited immediately from the association because the symbiont disposed of toxic organic acids. The metabolic arrangement in modern eukaryotic cells is similar, but now nutrients transferred to the mitochondrion include fatty acids, and the mitochondrion exports ATP.

Models of microbial syntrophies based on organic acid transfer have not been shown to work. Since most aerobic bacteria can use glucose directly, they are not dependent on other organisms to convert glu-

cose into organic acids. For example, aerobic bacteria were mutated so that they could not use glucose, but still could grow on lactate. The mutant cells were then placed in a co-culture with a fermentative "host" species, and glucose was provided. The mutant bacteria eventually re-evolved the capacity to use glucose directly. Once that happened, the bacterial cells grew more rapidly than the fermentative cells, which were eliminated from the co-culture. Thus, co-cultures based upon this type of organic acid transfer have not been stable.

Methanogenic syntrophy (Fig 5)

This has been called the "Hydrogen Hypothesis" [34]. A bacterial cell metabolized organic compounds to H_2 and CO_2 , and a second species converted those products into CH_4 . Over time, the methanogenic species (an Archeon) evolved into the nucleocytoplasm, and the H_2 -excreting bacteria became mitochondria. Several variations on this hypothesis have been suggested[35, 36].

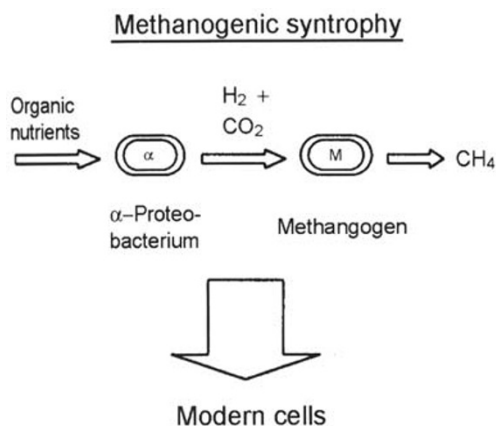


Fig 5. Mitochondrial origin from methanogenic syntrophy. The ancestor of the mitochondrion metabolized organic nutrients to H_2 and CO_2 . The Archeal methanogen used those products to make CH_4 . The methanogen eventually evolved into the eukaryotic nucleocytoplasm.

Criticisms of the "Methanogenic syntrophy" hypothesis are as follow. (1) Since methanogenic metabolism is incompatible with O_2 , methanogens are strict anaerobes, and not even facultative. Any step toward air-tolerance is lethal to a methanogen. There is no evolutionary path by which a methanogen can become aerobic, and there is no evidence that it ever happened. (2) Indications are that initially the eukaryotic cytoplasm was aerobic[18]. For example, the

cytoplasm contains numerous, apparently primitive O_2 -consuming enzymes, such as flavin-containing oxidases and membrane-bound b-cytochromes. For example, sterols, which are a diagnostic feature of eukaryotic cells, are formed from squalene by such a cytoplasmic oxygenase. (3) Methanogenesis is a specialized type of metabolism that requires several unique cofactors. No vestige of these cofactors has been found in any eukaryote, nor any other trace of methanogenic metabolism. (4) When eukaryotic cells are involved in a methanogenic association, such as occur in certain freshwater ciliates[30], the eukaryotic cell itself never produces methane, but instead uses symbiotic methanogenic microbes. The nucleocytoplasm never reverts to being a methanogen, suggesting that it never was one.

Sulfur Syntrophy (Fig 6)

A third type of syntrophy could be based upon sulfur. Mitochondria are closely related to purple sulfur bacteria, which oxidize H_2S either photosynthetically or by using O_2 . The nucleocytoplasm is related to basal Archea that probably reduced sulfur to H_2S [37]. In the hypothetical premitochondrial symbiosis, the bacterium oxidized H_2S to sulfur and the Archeon reduced it back to H_2S . Sulfur cycled repeatedly, in effect serving as an electron carrier between the two organisms[38].

Sulfur has been described as a "large, soft atom" that requires little activation energy[39]. It is particularly well suited for biological reduction and oxidation (redox) reactions, and frequently is used by organisms in both anoxic and aerobic environments. Sulfur is widely available on earth, including in the times before O_2 became abundant. In summary, sulfur is ideal for biological redox reactions, and was available in ancient times.

The way that sulfur can work is illustrated in the classic laboratory experiments of Wolfe and Pfennig [40]. They set up a co-culture of the photosynthetic green sulfur bacterium *Chlorobium* sp. with the heterotrophic sulfur-reducing bacterium *Sulfospirillum deleyianum*. In the laboratory cultures each species could grow only in the presence of the other. *Sulfospirillum* oxidized formic acid to CO_2 while reducing elemental sulfur to H_2S . *Chlorobium* reduced CO_2 to organic molecules, using H_2S as the electron

donor and oxidizing it back to elemental sulfur. The sulfur was required in only small, catalytic amounts because it cycled repeatedly between the two species.

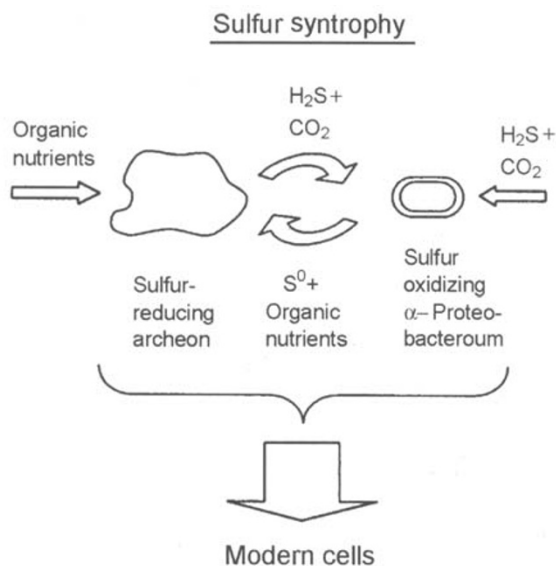


Fig 6. Mitochondrial origin from sulfur syntrophy. Sulfur was reduced to H₂S by an heterotrophic Archeon similar to *Thermoplasma*. An α-Proteobacterium oxidized the H₂S either photosynthetically or by using O₂. Sulfur cycled between the cells, and carbon accumulated.

A different example from nature is the close-knit bacterial consortium, "*Chlorochromatium aggregatum*" (Fig 7). This is 2 species of bacteria associated so tightly that they were described initially to be a single organism[41]. The consortium consists of a large central non-photosynthetic cell, now known to be a β-Proteobacterium, surrounded by about a dozen photosynthetic green sulfur bacteria[28]. This and similar consortia involving photosynthetic sulfur bacteria are highly successful, and occur worldwide in ponds that contain a lower H₂S-rich layer[42]. It has been questioned whether H₂S is transferred directly from species to species[43], but since the outer bacteria are obligate H₂S consumers, sulfide oxidation is certainly involved.

The criticism of the sulfur syntrophy model is that H₂S is highly toxic. It inhibits mitochondrial Respiratory Complex IV, and is equally as poisonous as cyanide[44]. Concentrations much greater than 1 mM sulfide inhibit the respiration of most eukaryotic cells. Thus, for sulfur to have a role in eukaryotic

cells, free sulfide concentrations should be kept below 1 μM.

Predictions of the "Sulfur syntrophy" hypothesis

If eukaryotic cells originated from a sulfur-based symbiosis, then one might expect vestiges of sulfur metabolism in modern cells. One indication of unsuspected sulfur metabolism was the enzyme "rhodanese", a sulfur transferase, known for years to be present in eukaryotic cells, but with no well-explained function[45].

When experimentally tested, eukaryotic cells were avid metabolizers of sulfur, both consuming H₂S and producing it. In mitochondria, H₂S is oxidized near Respiratory Complex III, possibly by a sulfide:quinone oxidoreductase that occurs widely throughout eukaryotes[46]. Oxidation of H₂S uses the electron transport chain, and can be coupled to ATP synthesis[47].

In the cytoplasm one finds sulfur reduction. When incubated in anoxic conditions with elemental sulfur, all eukaryotes tested have produced H₂S[48]. Human erythrocytes were a particularly interesting cell type because they have no mitochondria. When elemental sulfur was added to human red cells, H₂S was copiously produced, including even when O₂ was present [49].

Recent research in the laboratory has focused on the freshwater protozoan *Tetrahymena pyriformis*. As shown in Fig 8, this single-celled organism either produces H₂S or consumes it, depending upon the availability of O₂. In aerobic conditions, < 1 mM H₂S was consumed at about 1/3 the rate of O₂ consumption. When the cells were fractionated, H₂S consumption was associated entirely with the mitochondria. Sulfide consumption required the availability of O₂, and was inhibited by respiratory poisons such as CN⁻, confirming the involvement of the mitochondrial electron transport chain. In anoxic conditions *T. pyriformis* produced H₂S slowly from endogenous sources of sulfur, or rapidly when elemental sulfur was added. Following cell fractionation, the soluble cytoplasmic fraction produced H₂S even when aerated.

Observations such as these lend support to the sulfur syntrophy hypothesis. In modern cells H₂S is consumed by mitochondria, and H₂S is produced by the cytoplasm. The rates of sulfur metabolism are

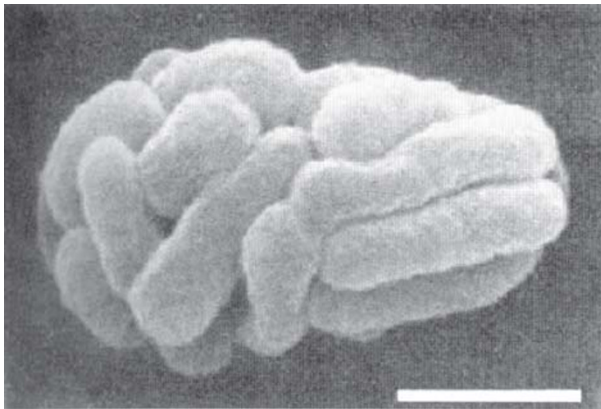


Fig 7. "Chlorochromatium aggregatum", an example of a consortium that uses H₂S. Sulfide-oxidizing photosynthetic bacteria surround a central heterotrophic cell. Hypothetically, the central cell transfers H₂S and CO₂ to the photosynthetic bacteria, which in return export sulfur and organic molecules back to the central cell. Scanning electron micrograph from ref [50]. Bar=1 μ m

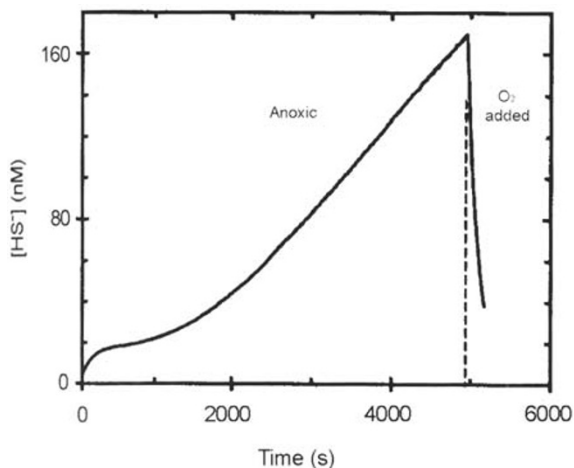


Fig 8. Both production and consumption of H₂S by the protozoan *Tetrahymena pyriformis*. Sulfide concentrations were measured using a sulfide-specific electrode. Initially the suspension was flushed with N₂ to remove O₂. At 4950 sec, 50 μ l of O₂ were injected into the cell suspension, shifting the cells from H₂S production to H₂S consumption. Details: cells were collected from a 2 day culture, washed, and resuspended in 0.075 M NaCl, 20 mM sodium 4-morpholinepropanesulfonate, and 1 mM sodium diethylenetriaminepentaacetate (pH 7.0). The experiment used cells amounting to 3.54 mg protein in 3 ml of buffer.

unexpectedly rapid, suggesting that sulfur metabolism may continue to be important today, perhaps as a shuttle for electrons.

CONCLUSIONS

There are several robust but contrasting hypotheses about the metabolic arrangement that existed in the ancient microbial symbiosis that gave origin to mitochondria. None of the hypotheses has been disproved, but the Sulfur Syntrophy model has resulted in novel predictions that have been tested and verified.

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