

The hydrogen hypothesis for the first eukaryote

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A new hypothesis for the origin of eukaryotic cells is proposed, based on the comparative biochemistry of energy metabolism. Eukaryotes are suggested to have arisen through symbiotic association of an anaerobic, strictly hydrogen-dependent, strictly autotrophic archaeobacterium (the host) with a eubacterium (the symbiont) that was able to respire, but generated molecular hydrogen as a waste product of anaerobic heterotrophic metabolism. The host's dependence upon molecular hydrogen produced by the symbiont is put forward as the selective principle that forged the common ancestor of eukaryotic cells.

Unicellular eukaryotes (protists) that possess neither mitochondria nor hydrogenosomes—the double-membrane-bounded, H_2 - and ATP-producing organelles of amitochondriate protists¹—have figured prominently in hypotheses for eukaryotic origins^{2–4}. Candidates for the most primitive contemporary eukaryotes have been sought among these groups, because they are eukaryotes but are devoid of organelles that descend from free-living eubacteria under the endosymbiont hypothesis⁵. Two main hypotheses currently explain how such a hypothetically ancestral, organelle-lacking eukaryote might have arisen. The Archezoa hypothesis^{6,7} is founded in comparative cytology. It posits that eukaryotes and archaeobacteria share a common ancestor, and that the ancestral eukaryote (an archezoan) arose directly from that stem by evolving a nucleus, a primitive cytoskeleton, and endocytosis. A descendant of that archezoan is suggested to have endocytosed a eubacterium that became the mitochondrion, while others remained amitochondriate. The alternative ‘fusion’ hypothesis draws primarily upon molecular phylogenetic data⁸. It accepts Archezoa as the starting point of eukaryotic evolution, but derives them from a fusion event between an archaeobacterium and a eubacterium. In some formulations, fusion involved engulfment of the archaeobacterium by the eubacterium, in other words an endosymbiotic origin of the nucleus^{9,10}. The resulting chimaera is proposed to have then evolved eukaryotic structures and acquired the mitochondrion as above.

The Archezoa hypothesis offers plausible and explicit mechanisms for the origin of eukaryotic cellular features, yet cannot directly account for the findings (1) that many eukaryotes that lack both mitochondria and hydrogenosomes possess nuclear genes thought to be of eubacterial origin^{11–15} and (2) that among contemporary archaeobacteria, none can be found that possesses cytological structures that can be meaningfully homologized to those typical of eukaryotes. The fusion hypothesis (and variants thereof³) accounts directly for eubacterial genes in Archezoa, yet offers no plausible explanation for the biological context of interkingdom fusion and fails to offer explicit mechanisms for the origin of eukaryotic structures other than the endoplasmic reticulum and nucleus.

Both hypotheses embrace the view that the host of mitochondrial symbiosis was a eukaryote. Neither hypothesis examines specifically what type of energy metabolism the ancestral eukaryote and its antecedent(s) may have had, but rather assume that the host was heterotrophic before the acquisition of mitochondria^{4,5,16,17}. Here we summarize energy metabolism in non-photosynthetic eukaryotes and put forward an explicit inference as to its ancestral state. The result of that inference is a hypothetical, primitive eukaryotic cell with surprising attributes.

Eubacterial energy metabolism in eukaryotes

Eukaryotes that do not possess functional plastids are heterotrophic: they satisfy their ATP needs through the oxidative breakdown of reduced organic compounds (Fig. 1). Glycolysis (the Embden–Meyerhoff pathway) is the backbone of eukaryotic energy metabolism: one mol glucose is oxidized to pyruvate with the help of NAD^+ with a net yield of 2 mol ATP. In mitochondriate eukaryotes, pyruvate is usually further oxidized in the mitochondria through the pyruvate dehydrogenase complex (PDH), the Krebs cycle and O_2 respiration to yield CO_2 and water under the production of an additional 34–36 mol ATP per mol glucose. Amitochondriate eukaryotes meet their energy needs through anaerobic fermentations^{18–21}. They also obtain 2 mol ATP from glycolysis, but they differ from mitochondriate eukaryotes with respect to the fate of pyruvate. In amitochondriate eukaryotes, pyruvate is metabolized through pyruvate:ferredoxin oxidoreductase^{18–21} (PFO), rather than through PDH. In eukaryotes that lack organelles involved in core metabolism (type I amitochondriate eukaryotes^{18–21}), cytosolic PFO decarboxylates pyruvate, yielding reduced ferredoxin and acetyl-CoA. The latter is converted into a mixture of ethanol and acetate, the relative amounts of which depend upon environmental conditions, yielding between 0 and 2 additional mol ATP per mol glucose (Fig. 1a). In amitochondriate eukaryotes that harbour hydrogenosomes (type II amitochondriate eukaryotes^{18–21}), cytosolic pyruvate is imported into the organelle, where PFO converts it to CO_2 , acetyl-CoA and reduced ferredoxin. Ferredoxin is reoxidized by hydrogenase, producing the H_2 characteristic of the organelle. Per mol glucose, pyruvate metabolism in hydrogenosomes yields two additional mol ATP and two mol each of H_2 , CO_2 and acetate as waste products (Fig. 1b).

Whereas the endosymbiont hypothesis readily accounts for the eubacterial ancestry of mitochondrial energy metabolism⁵, the evolutionary origin of energy metabolism in amitochondriate protists has been more elusive. But, as summarized below, recent data suggest that it, too, is of eubacterial origin (in contrast to the archaeobacterial ancestry presumed for various components of the eukaryotic genetic apparatus^{3,14,22–25}). Because molecular data indicate that hydrogenosomes and mitochondria share a common ancestor^{26–29}, and because PFO and other enzymes of hydrogenosomes are of eubacterial ancestry^{19,30}, a case can be made for a eubacterial origin of (at least major segments of) energy metabolism in type II amitochondriate protists. This view is furthermore supported by the findings that some contemporary proteobacteria³¹ and cyanobacteria³² (1) can grow aerobically or anaerobically, (2) possess respiratory chains and hydrogenase and (3) possess

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homologues of PDH and PFO, in turn suggesting that the common ancestor of hydrogenosomes and mitochondria did as well. Molecular data for enzymes of type I amitochondriate protists, such as PFO and bifunctional aldehyde/alcohol dehydrogenase in both *Giardia lamblia* and *Entamoeba histolytica* suggest that these enzymes are eubacterial, rather than archaeobacterial in origin¹⁵. Acetyl-CoA synthetase (ADP-forming) found in type I amitochondriate protists³³ is common among archaeobacteria³⁴, although some eubacteria are known that also possess this enzyme³³. Other nuclear genes of amitochondriate protists, in addition to those of PFO-related pathways, are thought to descend from eubacteria rather than from archaeobacteria^{11–14}. Notably, such genes include several enzymes of the glycolytic pathway from glucose to pyruvate in the eukaryotic cytosol^{12,13,19,35,36}.

At face value, these data suggest that (1) many, and probably all, groups of amitochondriate protists harboured eubacterial symbionts in their evolutionary past (2) that the enzymes essential to all three known types of eukaryotic energy metabolism were acquired from eubacteria and (3) that the free-living common ancestor of hydrogenosomes and mitochondria was capable of producing sufficient ATP both in anaerobic and aerobic environments. The simplest interpretation of these findings is that the three forms of energy metabolism found in eukaryotes today were inherited from the common ancestor of hydrogenosomes and mitochondria, which possessed the enzymes necessary to perform all three. From that it would follow that in the case of type II amitochondriate protists, the respiratory pathway and hydrogenosomal genome have been lost, whereas in the case of type I amitochondriate protists the entire organelle has additionally been lost. The phylogenetic distribution of type I and type II amitochondriate protists across ribosomal RNA phylogenies indicate that these losses have occurred many times in independent eukaryotic lineages^{3,7,19,21,23,37}. However, for the purposes of this paper, the order of these losses is irrelevant.

Metabolism in the context of symbiont origins

Traditional views on mitochondrial origins posit that their benefit to the host was increased efficiency of ATP production through respiratory carbohydrate breakdown. However, this generally accepted premise carries several tenuous corollary assumptions, most notably (1) that the host was unable to synthesize sufficient amounts of ATP by itself, (2) that the symbiont synthesized ATP in amounts exceeding its needs and (3) that the symbiont could export ATP to its environment, so that the host could realize this benefit. These phenomena are unknown among contemporary

cells, suggesting that ATP itself is unlikely as an initial symbiotic benefit. If not ATP, then what? Attempting to infer the context of benefit in an ancient symbiosis is necessarily speculative, but deserves exploration.

What might the symbiont have needed, what might have it been able to provide? From molecular phylogeny we can assume that it was a member of the α -proteobacteria^{5,14,26–29}, and that it therefore may have been photosynthetic or non-photosynthetic, autotrophic (able to satisfy its carbon needs from CO₂ alone) or heterotrophic, anaerobic or aerobic, or all of the above, as is the case for many contemporary representatives of the group, such as *Rhodobacter sphaeroides*³⁸. From the previous section, we posit that the symbiont possessed (at least) PDH, a Krebs acid cycle, a complete respiratory chain and all the enzymes for energy metabolism as are found in amitochondriate protists. In order to grow, such a bacterium needs reduced organic compounds, but has little to offer the host other than waste products of its metabolism: CO₂ in the case of aerobic respiration, CO₂, H₂ and acetate in the case of ‘hydrogenosomal’ metabolism. Thus, if the context of symbiosis was metabolic, there are two possibilities: the host either could have (1) provided benefit to the symbiont in the form of reduced carbon substrate, or (2) reaped benefit from waste products of the symbiont’s metabolism.

Alternative (1) is unlikely, because among the plethora of lithotrophic (generating ATP through redox reactions) and heterotrophic pathways known among contemporary archaeobacteria³⁴, only two produce reduced carbon compounds: heterotrophic fermentation and methanogenesis. Fermentation is unlikely as a benefit from host to symbiont, because if both grew heterotrophically, competition, not symbiosis would have ensued. Methane, by contrast, is the sole energy source of obligate methanotrophic α -proteobacteria³⁹. It is possible, but highly unlikely, that such was the initial context of host–symbiont association. This is because contemporary methanotrophy is strictly dependent upon molecular oxygen³⁹, whereas contemporary methanogens are strict anaerobes^{34,40}. An intimate cellular association of the type necessary to generate endosymbiosis cannot be construed, and has not been observed in natural communities⁴¹.

Alternative (2), however, unearths the many plausible benefits of hydrogen. Many archaeobacteria are strictly dependent upon H₂ for their ATP production^{34,40}. Moreover, for many methanogens (the strictly lithoautotrophic forms), H₂O and CO₂ are the sole source of both energy and carbon^{34,40}, whereas others can utilize alternative carbon sources such as methylamine, formic acid and acetate (all of which are waste products of eubacterial metabolism), and a few can grow on acetate alone^{37,40}. For methanogens, all three waste products

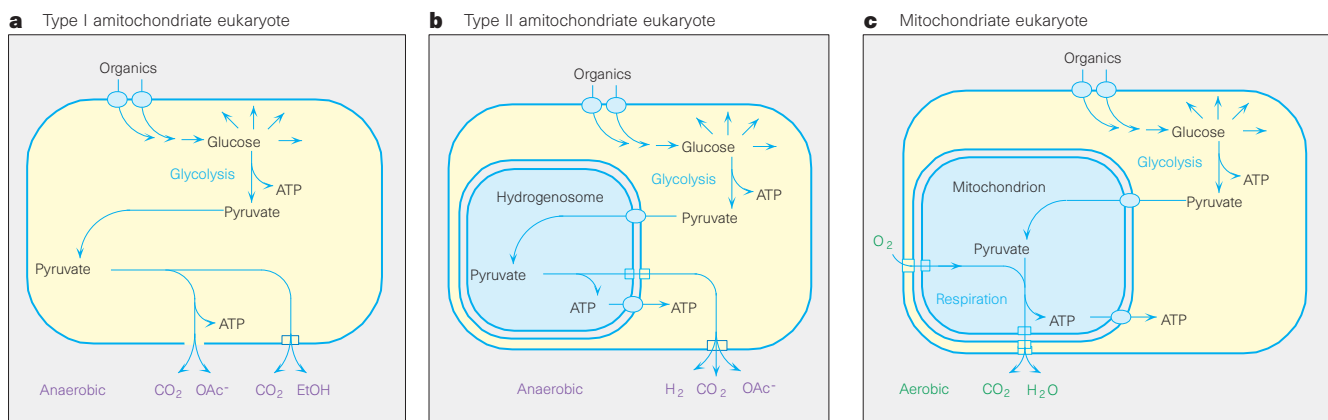


Figure 1 Schematic summary of forms of energy metabolism among heterotrophic eukaryotes (see refs 18 and 19 for details). OAc⁻, acetate; EtOH, ethanol; ATP, adenosine triphosphate. Respiration in the figure designates the combination of oxidative decarboxylation by PDH, Krebs cycle (also known as citric acid

cycle, tricarboxylic acid cycle, TCA cycle) to produce NADH + H⁺ and FADH₂, and the respiratory electron transport chain that donates electrons and protons to O₂, yielding ATP through oxidative phosphorylation.

of the symbiont's anaerobic metabolism are fuel for life. Furthermore, methanogens are strictly anaerobic, congruent with the conditions under which both hydrogenosomes and free-living eubacteria produce H_2 . This is intriguing, but is it realistic? Are associations between methanogens and hydrogen-producing organisms observable today? Yes, abundantly so. Anaerobic syntrophy between methanogens and hydrogen-producing organisms has been known for many years⁴² and has been studied in some detail^{37,43-45}. It is observed in marine sediments³⁷, deep in the Earth's crust⁴⁶, and fascinating examples are known in which endosymbiotic methanogens cling not to free-living eubacteria, but hydrogenosomes themselves in the cytosol of amitochondriate protists^{37,43-45}. The possibility that this type of symbiotic association may have been involved in the context of mitochondrial/hydrogenosomal origins is sufficiently intriguing to examine further.

Host dependence upon hydrogen: what happens?

Let us briefly explore a hypothetical symbiosis between a free-living, H_2 - and CO_2 -producing eubacterium (the symbiont) and a methanogenic archaeebacterium (the host). They would have to meet in anaerobic environments where CO_2 and geological H_2 are abundant, so that the host is viable from the start (Fig. 2a). But once the pair is physically removed from the H_2 source (by whatever means), the host becomes immediately and strictly dependent upon the heterotrophic eubacterial symbiont (Fig. 2b). This is conceptually satisfying, because it provides a strong selective force that irreversibly associates symbiont and host. If the symbiont escapes, the host starves immediately. Such hosts are thus most successful if they (1) stick tightly to symbionts and (2) can reap the greatest benefit from them. This could conceivably select host cell shapes of large surface area that tend to surround symbionts (not endocytose them, because archaeobacteria have no cytoskeleton), increasing contact, so that more H_2 and CO_2 could be filtered through the host's cytosol (Fig. 1).

As long as the symbiont finds sufficient organic substrates, this symbiosis of prokaryotes is indefinitely sustainable, but a limitation becomes evident. Host benefit from increased surface area to the symbiont concomitantly decreases the latter's ability to effervesce gaseous life into its host, because surface contact to the environment for fuelling its own metabolism (and producing hydrogen to fuel the host) is impaired. If competition for organic substrates occurs, so will selection for hosts that find a means of utilizing their own environmental surface to import fermentable organic substrates (something that contemporary methanogens cannot do^{34,40}) for the symbiont. This could be done by evolutionary invention of

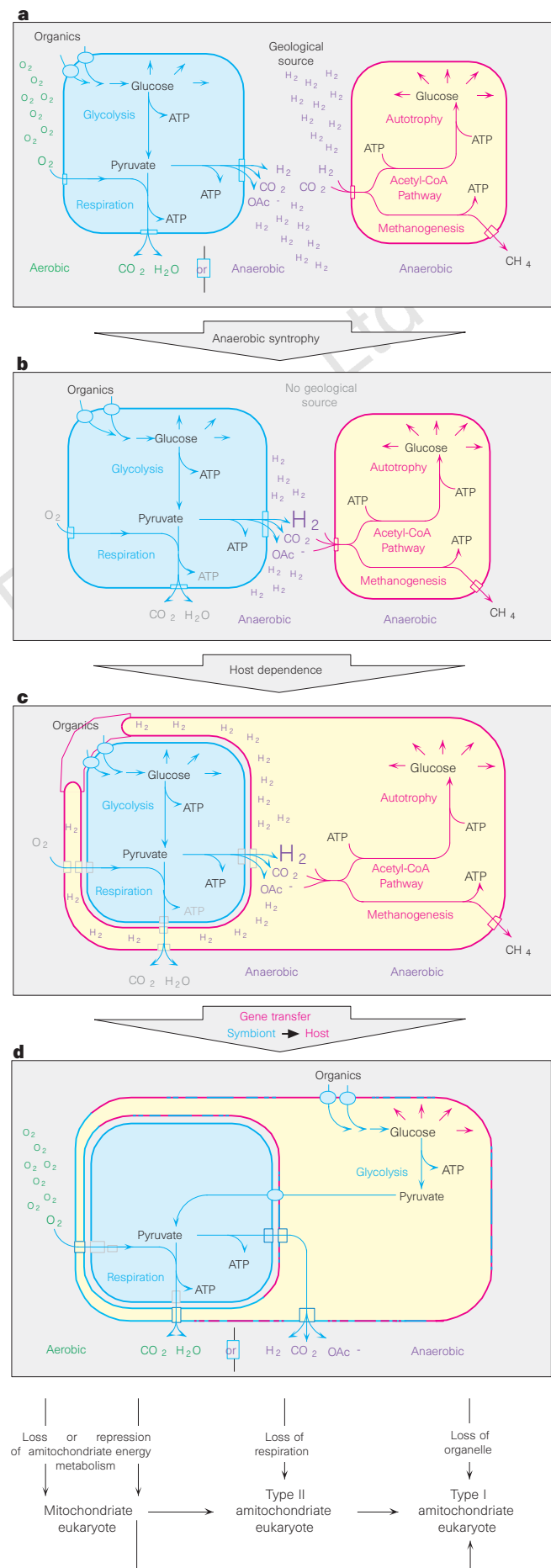


Figure 2 Hypothetical model to derive the ancestral state of eukaryotic energy metabolism put forward here, invoking strict dependence of the host upon waste products of the symbiont's anaerobic heterotrophy (see text). Host components are shaded red (cytosol yellow), symbiont components are shaded blue. The composite nature of membranes in **d** symbolizes the process of replacement of archaeobacterial lipids (glycerol ethers of isoprenes) with eubacterial lipids (glycerol esters of fatty acids) through loss of the host's lipid biosynthetic pathway. For an alternative explanation of the origin of eubacterial lipids in eukaryotes, see ref. 6. Anaerobic substrates and end products are indicated in purple, aerobic substrates and end products are indicated in green. Substrates and end products, the non-availability of which for a given step underly ecological factors, are indicated in grey.

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something that did not exist before the initial meeting (importers of reduced carbon in methanogens), or without invention, by merely genetically rearranging pre-existing components. If eubacterial genes for the symbiont's carbon importers are transferred by whatever mechanism to the archaeobacterial chromosomes of the host's cytosol, are expressed there, and if the products are functional in the archaeobacterial membrane, then the host would in principle be able to feed its symbiont with organics and thus feed itself with H₂ and CO₂ (and acetate, depending upon the capability of the host). This is neither outrageously improbable, nor does it involve an evolutionary invention. It merely requires the genetic systems of eubacterium and archaeobacterium to be sufficiently compatible as to allow expression of the transferred gene(s). Such genetic compatibility may be less today than it was two or three billion years ago, at which time symbiont and host may have shared a common ancestor only one or two billion years before. Furthermore, the type of endosymbiotic gene transfer invoked here, that is, without return of the gene product to the cell compartment that donated the gene, is well documented among contemporary eukaryotes^{37,47}.

But importers alone do not allow the host to feed its symbiont. This is because, in contrast to the heterotrophic metabolism of the symbiont that generates ATP from carbohydrates, the autotrophic metabolism of the host is specialized towards synthesizing carbohydrates from CO₂ at the expense of ATP gained by other means^{34,40}. As a consequence, imported carbon flows in the wrong direction: host and symbiont alike will starve unless carbon flux to the symbiont is established, providing selection for the latter to occur. To achieve this, either (1) the host's carbohydrate metabolism must acquire, step-by-step, the regulatory properties necessary to make it run backwards (a series of evolutionary inventions), or (2) the symbiont's carbohydrate metabolism must simply be transferred to the cytosol, again through straight endosymbiotic gene transfer (single-step relocation of pre-existing components). This still does not completely solve the problem, because two pathways of carbon metabolism are now running in opposite directions (catabolic and anabolic) in the same cytosol. The result is futile cycling (glucose + ADP → C compounds + ATP → glucose + ADP), and selection dictates that one of these pathways must be eliminated. But only if the host's pathway is eliminated can the symbiosis survive (Fig. 2d).

This leads to a curious situation. The selective pressure that associated the partners from the start and that drove the integration of eubacterial genes into archaeobacterial chromosomes was the host's strict dependence upon hydrogen produced by the symbiont. But by transferring the symbiont's importers and glycolysis to the cytosol in order to satisfy that dependence, the host suddenly can meet both its carbon and energy needs from organic substrates. The functions of both methanogenesis and autotrophy have been replaced, and there is no obvious selective pressure to retain either. The host has irreversibly become heterotrophic, and hydrogen is once again a waste product, but now of a compartmentalized metabolism.

Quite surprisingly, the result of this effortless metabolic endeavour is a hydrogenosome with a genome in an archaeobacterial host with cytosolic chromosomes, a cell that is organized in a manner strikingly similar to the amitochondriate eukaryote *Trichomonas vaginalis*¹. That this hypothetical primitive eukaryote does not possess a nuclear membrane is not disturbing; the hydrogen hypothesis simply derives a different stage of the eukaryotic cell cycle (open mitosis) than previous hypotheses do. Not a single evolutionary invention was necessary to deduce this organelle-bearing cell.

One principle, two symbioses: O₂ and plastids

Once the host's metabolic dependence upon hydrogen vanished, so would its confinement to anaerobic habitats. As outlined in previous sections, the symbiont would also have been able to respire by

virtue of its pre-existing metabolic diversity, hence O₂ could have then become advantageous to the eukaryote through respiratory ATP synthesis (and through the invention of an ATP transporter to allocate it to the cytosol). But importantly, utilization of O₂ would have been a true advantage, not a dependence as in the case of H₂ suggested here to have established the heterotrophic organelle. Such O₂-utilization could have occurred with global increases of atmospheric O₂ levels roughly 2 billion years ago⁴⁸, or conceivably could have entailed syntrophy of the eukaryote with O₂-producing cyanobacteria, or both, but probably in independent lineages of a young but diversified eukaryotic kingdom.

The foregoing entails the assumption that the symbiont's respiration machinery (Krebs cycle and oxidative phosphorylation) was not lost during the H₂-dependent phases of symbiosis, and hence the assumption that it was maintained by selection. To account for this, we suggest that the respiratory pathway of the symbiont might have enabled the anaerobic cell to free its environment of oxygen, as contemporary amitochondriate protists do (albeit by other means¹⁹). It is less evident why genes for proteins specific to energy metabolism in amitochondriate protists, such as cytosolic and hydrogenosomal PFO, have been preserved throughout the evolution of mitochondriate eukaryotes, so as to be readily recruitable in multiple lineages during the reversion to anaerobic energy metabolism, as has been clearly demonstrated among the anaerobic ciliates⁴⁴. To account for this, we offer that such enzymes perform(ed) additional essential functions for eukaryotic cells, the biochemistry of which is unknown. In support of this view are the findings that (1) dual functions for PFO in eubacteria exist, where it is an integral component of the nitrogen fixation machinery³⁰ (*nifH*) by virtue of its powerful electron-donating potential, and (2) that a homologue of PFO is encoded within the yeast genome. In eukaryotes, no dual function for PFO is yet known, although it does substitute for PDH in some mitochondria, for example in *Euglena*³⁰.

The notion that syntrophy may have associated a cyanobacterial symbiont with its heterotrophic, eukaryotic host is attractive, because it would entail simple reiteration of the same principle suggested for the origin of the heterotrophic symbiont, and only the beneficial waste product of the symbiont's metabolism (O₂) is different. Thus, the contemporary benefits that both mitochondria (respiration) and plastids (photoautotrophy) confer upon their hosts may be very different and much more complex than the benefit initially provided by either (waste H₂ and O₂). A similar grade, in which biological complexity is born of chemical simplicity, has been suggested for the evolution of metabolism itself¹⁷. However, the hypothesis that one organelle may have arisen through syntrophic association does not bear on views concerning the origin of the other: the reasoning is similar but the specific premises are independent.

Conclusion

The hydrogen hypothesis can readily account for the origins of eukaryotic energy metabolism by invoking differential loss from an explicitly derived ancestral state (Fig. 2d). In doing so, it furthermore accounts for the origin of a basic eukaryotic cell in a manner that differs substantially from previous views on the topic. First, this hypothesis posits that the origins of the heterotrophic organelle (the symbiont) and the origins of the eukaryotic lineage are identical. Second, it demands only three properties of the host: (1) that it was anaerobic, (2) that it possessed strictly hydrogen-dependent metabolism and (3) that it was strictly autotrophic. It does not require the host to have possessed either nucleus, cytoskeleton, endocytosis or mitosis, therefore no organizational cline in the host lineage before the acquisition of the symbiont must be postulated. Third, it specifically posits a lethal selective force that irreversibly binds one symbiotic partner to the other. Hydrogen is the key. It is the bond that forges eukaryotes out of prokaryotes.

The archaeobacterial nature of the eukaryotic genetic apparatus

and the eubacterial nature of eukaryotic energy metabolism are premises that can be explained, not predictions that are fulfilled under this hypothesis. For both apparatuses, some exceptions to the rule can be expected—archaeobacterial transketolase in some eukaryotes³⁶ may be an example—and there is no obvious reason to expect either a eubacterial or an archaeobacterial origin for intermediate eukaryotic metabolism. Our hypothesis does not explain fundamental differences in prokaryotic and eukaryotic genome organization^{5,49}, and it does not explain the origin of eukaryotic structures that have been the focus of previous views. We also stress that the hypothetical process outlined in Fig. 2 in no way precludes the possibility that the host may have possessed a cytoskeleton before its association with the symbiont. However, we posit that the host was autotrophic: the selective advantages conferred by a cytoskeleton—arguably a prerequisite for phagocytotic feeding⁶—are less evident for an autotroph than they are for the compartmentalized heterotroph inferred here. That cell has time, energy and ample genetic starting material (two highly divergent and partially merged prokaryotic genomes) to evolve cytological and genetic traits that are specific to the eukaryotic lineage.

A methanogenic ancestry of the host is only one of several possible H₂-dependent scenarios. One in which an autotrophic host used H₂ as an electron donor, but electron acceptors other than CO₂ (sulphurous compounds, for example³⁴) could be elaborated by the same logic, whereby the host so deduced would also have been dependent upon such compounds, rather than solely upon its heterotrophic symbiont. Yet methanogenesis is attractive as the host's metabolism for several reasons. (1) It can be traced sufficiently deep into archaeobacterial phylogeny⁵⁰ as to be a candidate for a pathway ancestral to the kingdom. (2) No methanogen is known that is heterotrophic; those that utilize acetate and/or reduced C₁ compounds do so for methanogenesis and autotrophy^{34,40}. (3) Methanogens are strictly anaerobic, and (4) can utilize all three products^{34,37,40} of the symbiont's anaerobic metabolism. (5) Widespread syntrophic association between methanogens and hydrogenosomes is observable^{37,43–45}. By the criteria of simplicity under competing alternatives and of explaining unknowns in terms of known quantities, methanogenesis fares well under Occam's razor.

This hypothesis generates numerous testable predictions. We firmly predict that evidence for a strictly H₂-dependent ancestry, and most probably a methanogenic ancestry of the host should ultimately be revealed by comparative genomics. In photosynthetic eukaryotes, we predict that fewer genes of archaeobacterial ancestry should be observable, because an additional eubacterial genome is incorporated into the cell, allowing endosymbiotic gene replacement further opportunity to eliminate functionally redundant, pre-existing archaeobacterial homologues³⁶. Finally, we predict that anaerobic heterotrophic habitats devoid of geological hydrogen may harbour eukaryotes more primitive than known forms, the metabolism of which should be accountable for under the premises stated here. □

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