The hydrogen hypothesis for the first eukaryote

William Martin* **& Miklós Müller†**

** Institut für Genetik, Technische Universität Braunschweig, Spielmannstrasse 7, D-38023 Braunschweig, Germany*
† The Rockefeller University, 1230 York Avenue, New York, New York 10021, USA

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 archaebacterium (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 protists[1]—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 hypothesis[5]. 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 archaebacteria share a common ancestor, and that the ancestral eukaryote (an archezoon) arose directly from that stem by evolving a nucleus, a primitive cytoskeleton, and endocytosis. A descendant of that archezoon is suggested to have endocytosed a eubacterium that became the mitochondrion, while others remained amitochondriate. The alternative 'fusion' hypothesis draws primarily upon molecular phylogenetic data[8]. It accepts Archezoa as the starting point of eukaryotic evolution, but derives them from a fusion event between an archaebacterium and a eubacterium. In some formulations, fusion involved engulfment of the archaebacterium 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 archaebacteria, none can be found that possesses cytological structures that can be meaningfully homologized to those typical of eukaryotes. The fusion hypothesis (and variants thereof[3]) 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 eubacteral ancestry of mitochondrial energy metabolism[5], 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 archaebacterial 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 proteobacteria[31] and cyanobacteria[32] (1) can grow aerobically or anaerobically, (2) possess respiratory chains and hydrogenase and (3) possess

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 archaebacterial in origin[15]. Acetyl-CoA synthetase (ADP-forming) found in type I amitochondriate protists[33] is common among archaebacteria[34], although some eubacteria are known that also possess this enzyme[33]. Other nuclear genes of amitochondriate protists, in addition to those of PFO-related pathways, are thought to descend from eubacteria rather than from archaebacteria[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 CO_2 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: CO_2 in the case of aerobic respiration, CO_2, H_2 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 archaebacteria[34], 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-proteobacteria[39]. 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 oxygen[39], 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 communities[41].

Alternative (2), however, unearths the many plausible benefits of hydrogen. Many archaebacteria are strictly dependent upon H_2 for their ATP production[34,40]. Moreover, for many methanogens (the strictly lithoautotrophic forms), H_2O and CO_2 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

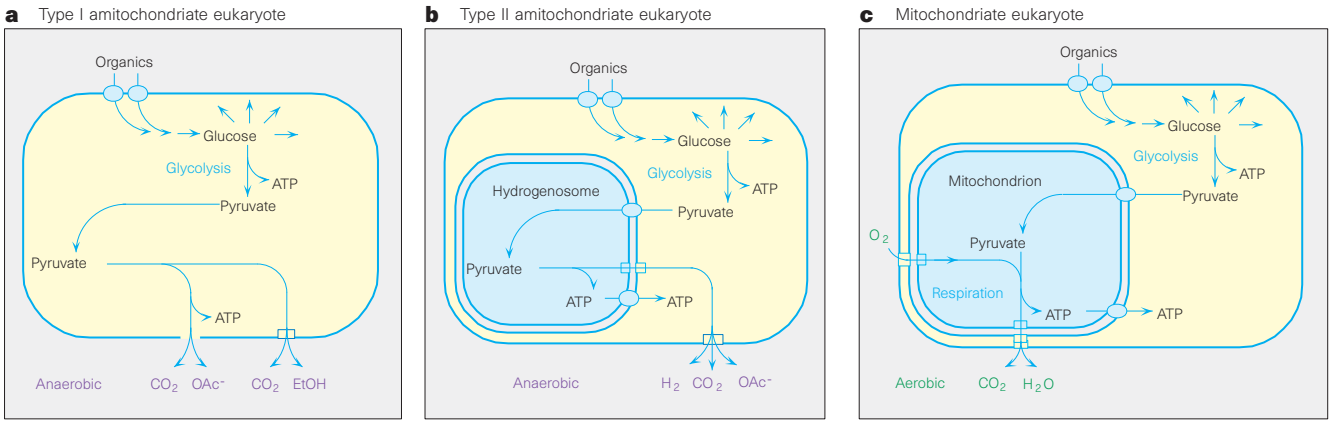

Figure 1 Schematic summary of forms of energy metabolism among heterotrophic eukaryotes (see refs 18 and 19 for details). OAc^-, acetate; EtOH, ethanol; ATP, adenosine triphosphate. Respiration in the figure designates the combination of oxidative decarobxylation by PDH, Krebs cycle (also known as citric acid cycle, tricarboxylic acid cycle, TCA cycle) to produce NADH + H^+ and $FADH_2$, and the respiratory electron transport chain that donates electrons and protons to O_2, 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 years[42] and has been studied in some detail[37,43–45]. It is observed in marine sediments[37], deep in the Earth's crust[46], 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 archaebacterium (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 archaebacteria 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

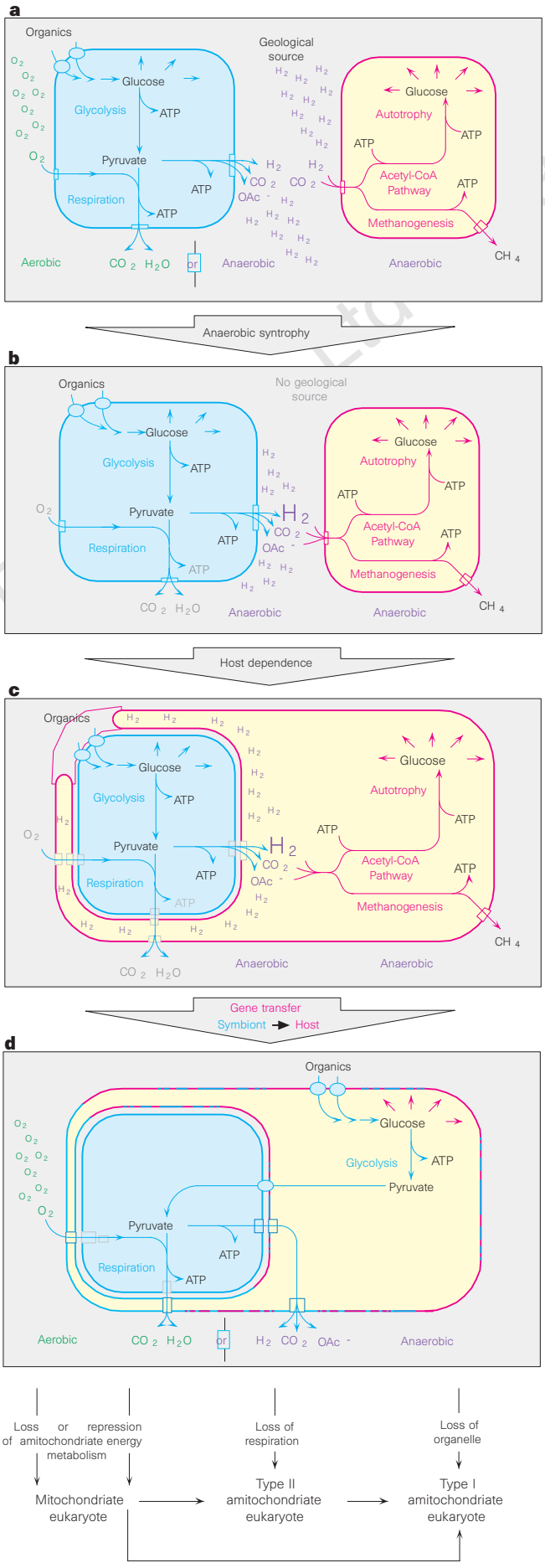

Figure 2 Hypothetical model to derive the ancestral state of eukaryotic energy metabolism put forward here, invoking strict dependence of the host upon waste products of the symbiont's anaerobic heterotrophy (see text). Host components are shaded red (cytosol yellow), symbiont components are shaded blue. The composite nature of membranes in **d** symbolizes the process of replacement of archaebacterial 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.

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 archaebacterial chromosomes of the host's cytosol, are expressed there, and if the products are functional in the archaebacterial membrane, then the host would in principle be able to feed its symbiont with organics and thus feed itself with H_2 and CO_2 (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 archaebacterium 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 CO_2 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 archaebacterial 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 archaebacterial 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: O_2 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 O_2 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 O_2 would have been a true advantage, not a dependence as in the case of H_2 suggested here to have established the heterotrophic organelle. Such O_2-utilization could have occurred with global increases of atmospheric O_2 levels roughly 2 billion years ago[48], or conceivably could have entailed syntrophy of the eukaryote with O_2-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 H_2-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 means[19]). 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 ciliates[44]. 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 machinery[30] (*nifJ*) 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 (O_2) 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 H_2 and O_2). A similar grade, in which biological complexity is born of chemical simplicity, has been suggested for the evolution of metabolism itself[17]. 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 archaebacterial 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—archaebacterial transketolase in some eukaryotes[36] may be an example—and there is no obvious reason to expect either a eubacterial or an archaebacterial 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 feeding[6]—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 H_2-dependent scenarios. One in which an autotrophic host used H_2 as an electron donor, but electron acceptors other than CO_2 (sulphurous compounds, for example[34]) 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 archaebacterial phylogeny[50] 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 C_1 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 H_2-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 archaebacterial 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 archaebacterial homologues[36]. 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. □

1. Müller, M. The hydrogenosome. *J. Gen. Microbiol.* **139,** 2879–2889 (1993).
2. Cavalier-Smith, T. Eukaryotes with no mitochondria. *Nature* **326,** 332–333 (1987).
3. Sogin, M. L., Silberman, J. D., Hinkle, G. & Morrison, H. G. Problems with molecular diversity in the Eukarya. *Symp. Soc. Gen. Microbiol.* **54,** 167–184 (1996).
4. Whatley, J. M., John, P. & Whatley, F. R. From extracellular to intracellular: the establishment of mitochondria and chloroplasts. *Proc. R. Soc. Lond. B* **204,** 165–187 (1979).
5. Gray, M. W. & Doolittle, W. F. Has the endosymbiont hypothesis been proven? *Microbiol. Rev.* **46,** 1–42 (1982).
6. Cavalier-Smith, T. The origin of eukaryote and archaebacterial cells. *Ann. NY Acad. Sci.* **503,** 7–54 (1987).
7. Cavalier-Smith, T. & Chao, E. E. Molecular phylogeny of the free-living archaezoan *Trepomonas agilis* and the nature of the first eukaryote. *J. Mol. Evol.* **43,** 551–562 (1996).
8. Zillig, W. *et al*. Did eukaryotes originate by a fusion event? *Endocytobiosis Cell Res.* **6,** 1–25 (1989).
9. Gupta, R. S. & Golding, G. B. The origin of the eukaryotic cell. *Trends Biochem. Sci.* **21,** 166–171 (1996).
10. Lake, J. A. & Rivera, M. C. Was the nucleus the first endosymbiont? *Proc. Natl Acad. Sci. USA* **91,** 2880–2881 (1994).
11. Clark, C. G. & Roger, A. J. Direct evidence for secondary loss of mitochondria in *Entamoeba histolytica*. *Proc. Natl Acad. Sci. USA* **92,** 6518–6521 (1995).
12. Henze, K. *et al*. A nuclear gene of eubacterial origin in *Euglena* reflects cryptic endosymbioses during protist evolution. *Proc. Natl Acad. Sci. USA* **92,** 9122–9126 (1995).
13. Keeling, P. W. & Doolittle, W. F. Evidence that eukaryotic triosephosphate isomerase is of alpha-proteobacterial origin. *Proc. Natl Acad. Sci. USA* **94,** 1270–1275 (1997).
14. Doolittle, W. F. Some aspects of the biology of cells and their possible evolutionary significance. *Symp. Soc. Gen. Microbiol.* **54,** 1–21 (1996).
15. Rosenthal, B. *et al*. Evidence for the bacterial origin of genes encoding fermentation enzymes of the amitochondriate protozoan parasite *Entamoeba histolytica*. *J. Bacteriol.* **179,** 3736–3745 (1997).
16. Searcy, D. G. in *The Origin and Evolution of the Cell* (eds Hartman, H. & Matsuno, K.) 47–78 (World Scientific, Singapore, 1992).
17. de Duve, C. *Blueprint for a Cell: the Nature and Origin of Life* (Patterson, Burlington, NC, 1991).
18. Coombs, G. H. & Müller, M. in *Biochemistry and Molecular Biology of Parasites* (eds Marr, J. J. & Müller, M.) 33–47 (Academic, London, 1995).
19. Müller, M. in *Evolutionary Relationships Among Protozoa* (eds Coombs, G. H., Vickermann, K., Sleigh, M. A. & Warren, A.) 109–132 (Chapman Hall, London, 1998).
20. Müller, M. Energy metabolism of protozoa without mitochondria. *Annu. Rev. Microbiol.* **42,** 465–488 (1988).
21. Müller, M. in *Christian Gottfried Ehrenberg-Festschrift anläßlich der 14. Wissenschaftlichen Jahrestagung der Deutschen Gesellschaft für Protozoologie*, 9.-11. Marz 1995 in Delitzsch (Sachsen) (eds Schlegel, M. & Hausmann, K.) 63–76 (Leipziger Universitätsverlag, Leipzig, 1996).
22. Iwabe, N., Kuma, K.-I., Hasegawa, M., Osawa, S. & Miyata, T. Evolutionary relationship of archaebacteria, eubacteria and eukaryotes inferred from phylogenetic trees of duplicated genes. *Proc. Natl Acad. Sci. USA* **86,** 9355–9359 (1989).
23. Woese, C., Kandler, O. & Wheelis, M. L. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria and Eukarya. *Proc. Natl Acad. Sci. USA* **87,** 4576–4579 (1990).
24. Langer, D., Hain, J., Thuriaux, P. & Zillig, W. Transcription in Archaea: similarity to that in Eukarya. *Proc. Natl Acad. Sci. USA* **92,** 5768–5772 (1995).
25. Yamamoto, A., Hashimoto, T., Asaga, E., Hasegawa, M. & Goto, N. Phylogenetic position of the mitochondrion-lacking protozoan *Trichomonas tenax*, based on amino acid sequences of elongation factors 1-α and 2. *J. Mol. Evol.* **44,** 98–105 (1997).
26. Horner, D. S., Hirt, R. P., Kilvington, S., Lloyd, D. & Embley, T. M. Molecular data suggest an early acquisition of the mitochondrion endosymbiont. *Proc. R. Soc. Lond. B* **263,** 1053–1059 (1996).
27. Bui, E. T. N., Bradley, P. J. & Johnson, P. J. A common evolutionary origin for mitochondria and hydrogenosomes. *Proc. Natl Acad. Sci. USA* **93,** 9651–9656 (1996).
28. Germot, A., Philippe, H. & Le Guyader, H. Presence of a mitochondrial-type 70-kDa heat shock protein in *Trichomonas vaginalis* suggests a very early mitochondrial endosymbiosis in eukaryotes. *Proc. Natl Acad. Sci. USA* **93,** 14614–14617 (1996).
29. Roger, A. J., Clark, C. G. & Doolittle, W. F. A possible mitochondrial gene in the early-branching amitochondriate protist *Trichomonas vaginalis*. *Proc. Natl Acad. Sci. USA* **93,** 14618–14622 (1996).
30. Hrdý, I. & Müller, M. Primary structure and eubacterial relationships of the pyruvate : ferredoxin oxidoreductase of the amitochondriate eukaryote, *Trichomonas vaginalis*. *J. Mol. Evol.* **41,** 388–396 (1995).
31. Blattner, F. R. *et al*. The complete genome sequence of *Escherichia coli* K-12. *Science* **277,** 1453–1474 (1997).
32. Kaneko, T. *et al*. Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. *DNA Res.* **3,** 109–136 (1996).
33. Sánchez, L. B. & Müller, M. Purification and characterization of the acetate forming enzyme, acetyl-CoA synthetase (ADP-forming) from the amitochondriate protist, *Giardia lamblia*. *FEBS Lett.* **378,** 240–244 (1996).
34. Schönheit, P. & Schäfer, T. Metabolism of hyperthermophiles. *World. J. Microbiol. Biotechnol.* **11,** 26–57 (1995).
35. Markoš, A., Miretsky, A. & Müller, M. A glyceraldehyde-3-phosphate dehydrogenase with eubacterial features in the amitochondriate eukaryote *Trichomonas vaginalis*. *J. Mol. Evol.* **37,** 631–643 (1993).
36. Martin, W. & Schnarrenberger, C. The evolution of the Calvin cycle from prokaryotic to eukaryotic chromosomes: a case study of functional redundancy in ancient pathways through endosymbiosis. *Curr. Genet.* **32,** 1–18 (1997).
37. Fenchel, T. & Finlay, B. J. *Ecology and Evolution in Anoxic Worlds* (Oxford Univ. Press, Oxford, 1995).
38. Gibson, J. L. & Tabita, F. R. The molecular regulation of the reductive pentose phosphate pathway in proteobacteria and cyanobacteria. *Arch. Microbiol.* **166,** 141–150 (1996).
39. Murrel, J. C. Genetics and molecular biology of methanotrophs. *FEMS Microbiol. Lett.* **88,** 233–248 (1992).
40. Thauer, R. K., Hedderich, R. & Fischer, R. in *Methanogenesis: Ecology, Physiology, Biochemistry and Genetics* (ed. Ferry, J. G.) 209–252 (Chapman & Hall, New York, 1993).
41. Conrad, R. Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2, and NO). *Microbiol. Rev.* **60,** 609–640 (1996).
42. Bryant, M. P., Wolin, E. A., Wolin, M. J. & Wolfe, R. S. *Methanobacillus omelianskii*, a symbiotic association of two species of bacteria. *Arch. Microbiol.* **59,** 20–31 (1967).
43. Broers, C. A. M., Stumm, C. K., Vogels, G. D. & Brugerolle, G. *Psalteriomonas lanterna* gen. nov., sp. nov., a free living amoboflagellate isolated from freshwater anaerobic sediments. *Eur. J. Protistol.* **25,** 369–380 (1990).
44. Embley, T. M. *et al*. Multiple origins of anaerobic ciliates with hydrogenosomes within the radiation of aerobic ciliates. *Proc. R. Soc. Lond. B* **262,** 87–93 (1995).
45. Finlay,k B. J., Embley, T. M. & Fenchel, T. A new polymorphic methanogen, closely related to *Methanocorpusculum parvum*, living in stable symbiosis within the anaerobic ciliate *Trimyema* sp. *J. Gen. Microbiol.* **139,** 371–378 (1993).
46. Stevens, T. O. & McKinley, J. P. Lithoautotrophic microbial ecosystems in deep basalt aquifers. *Science* **270,** 450–454 (1995).
47. Brinkmann, H. & Martin, W. Higher plant chloroplast and cytosolic 3-phosphoglycerate kinases: a case of endosymbiotic gene replacement. *Plant. Mol. Biol.* **30,** 65–75 (1996).
48. Kasting, J. F. Earth's early atmosphere. *Science* **259,** 920–926 (1993).
49. Poole, A. M., Jeffares, D. C. & Penny, D. The path from the RNA world. *J. Mol. Evol.* **46,** 1–17 (1998).
50. Rospert, S. *et al*. Methyl-coenzyme M reductase and other enzymes involved in methanogenesis from CO_2 and H_2 in the extreme thermophile *Methanopyrus kandleri*. *Arch. Microbiol.* **156,** 49–55 (1991).

Acknowledgements. We thank H. Brinkmann, M. Embley, K. Henze, R. Herrmann, R. Hensel, D. Oesterheld and L. Sánchez for critical comments on the manuscript and gratefully acknowledge financial support from the Deutsche Forschungsgemeinschaft (W.M.) and the National Institutes of Health (M.M.).

Correspondence should be addressed to W.M. (e-mail: w.martin@tu-bs.de) or to M.M. (e-mail: mmuller@rockvax.rockefeller.edu).