IUBMB *Life*, 55(4–5): 193–204, April–May 2003

Taylor & Francis
healthsciences

Review Article

Early Cell Evolution, Eukaryotes, Anoxia, Sulfide, Oxygen, Fungi First (?), and a Tree of Genomes Revisited

William Martin, Carmen Rotte, Meike Hoffmeister, Ursula Theissen, Gabriel Gelius-Dietrich, Simone Ahr and Katrin Henze

Institute of Botany III, University of Düsseldorf, Universitätsstr. 1, D-40225 Düsseldorf, Germany

Summary

Genomes contain evidence for the history of life and furthermore contain evidence for lateral gene transfer, which was an important part of that history. The geological record also contains evidence for the history of life, and newer findings indicates that the Earth's oceans were largely anoxic and highly sulfidic up until about 0.6 billion years ago. Eukaryotes, which fossil data indicate to have been in existence for at least 1.5 billion years, must have therefore spent much of their evolutionary history in oxygen-poor and sulfide-rich environments. Many eukaryotes still inhabit such environments today. Among eukaryotes, organelles also contain evidence for the history of life and have preserved abundant traces of their anaerobic past in the form of energy metabolic pathways. New views of eukaryote phylogeny suggest that fungi may be among the earliest-branching eukaryotes. From the standpoint of the fungal feeding habit (osmotrophy rather than phagotrophy) and from the standpoint of the diversity in their ATP-producing pathways, a eukaryotic tree with fungi first would make sense. Because of lateral gene transfer and endosymbiosis, branches in the tree of genomes intermingle and occasionally fuse, but the overall contours of cell history nonetheless seem sketchable and roughly correlateable with geological time.

IUBMB *Life*, 55: 193–204, 2003

Keywords Mitochondria; Hydrogenosomes; Endosymbiosis; Lateral gene transfer; Anaerobiosis; Sulfide; Anoxia; Divergence times.

INTRODUCTION

In an ideal world, the analysis of genome sequences would have fully uncovered the history of life by now. But as it stands, genome sequencing has mostly uncovered that humans can efficiently sequence genomes. Ten years ago, many imagined that a golden age of molecular evolution would emerge from genomics—an era of genome phylogenies in which the position of all organisms great and small was fully resolved in a unified tree of evolutionary history. In an ideal world, the genes of all genomes would be related by one and the same bifurcating process, combining all these genes into one grand alignment would produce the ultimate tree, biology's key to the past, a genome-enabled-time machine. That tree would have resolved all the branches and issues about which evolutionary biologists and systematists had ever quibbled. It would have put all organisms with a sequenced genome in their proper place in the larger scheme of things and would have allowed biologists to go about the enjoyable business of mapping out the evolution of morphological and biochemical characters among those lineages.

But genomes have not uncovered an ideal world. They have uncovered abundant evidence for horizontal gene transfer among prokaryotes (*1*) and they have uncovered abundant evidence for chimaerism in eukaryotes (*1–3*). Eukaryotes possess a mixture of genes, some of which clearly reflect a eubacterial ancestry, some of which clearly reflect an archaebacterial ancestry. Various eubacteria and archaebacteria also possess mixtures of genes that they have acquired and passed on both to their progeny and to various casual acquaintances from distant prokaryotic taxa via horizontal gene transfer. All genomes studied contain substantial numbers of genes which lack easily identifiable homologues among other lineages; these might be lineage-specific gene inventions, or fast-evolving genes that have simply lost the trace of their origin, or both.

Putting specific numbers on the amount of lateral gene transfer (LGT) that has occurred in the evolution of individual prokaryotic genomes is no easy matter. Case studies suggest that the fraction of horizontally transferred genes in genomes is substantial (*1–6*), estimates reaching up to 30% in some cases (*7*) or even more (*3*). At the same time, phylogeneticists

Received 3 April 2003; accepted 9 May 2003

Address correspondence to William Martin, Institute of Botany III, University of Düsseldorf, Universitätsstr. 1, D-40225 Düsseldorf, Germany; E-mail: w.martin@uni-duesseldorf.de.

Abbrevations: LGT, lateral gene transfer; Ga, billion years before present; PAL, present atmospheric levels; ATP, adenosine triphosphate; CoA, coenzyme A

ISSN 1521-6543 print/ISSN 1521-6551 online © 2003 IUBMB
DOI: 10.1080/1521654031000141231

are warning that many claims for horizontal gene transfer may largely reflect our inability to properly reconstruct the evolution of genes (*8*), or our poor sampling of critical lineages (*1, 9*), or both (*10*). Notwithstanding the difficulties of properly quantifying it, LGT does exist.

Although LGT does not dash all hopes of piecing together life's early history, it does make the puzzle of cell evolution much more difficult to reconstruct from the standpoint of genomes. But that is not completely bad news. It is also good news for biologists, because it makes it all the more important to look for independent evidence for life's history, for example in the geological record in the form of fossils or isotopic evidence. Among the eukaryotes, it also prompts the search to identify major endosymbiotic events that can help to define assemblages of related cell lineages (and their ancestor cell lineages).

The purpose of this paper is to link up various kinds of mutually consistent evidence for early cell evolution in such a way as to present a general schematic view of life's unicellular history. Many might think that the 'universal' rRNA tree already does that, but the rRNA tree describes the evolution of only one gene and therefore cannot accomodate or depict LGT. Furthermore the rRNA tree is a strictly bifurcating tree, yet the evolution of eukaryotes entails the symbiotic origins of mitochondria and of plastids (both primary and secondary). In endosymbiosis, two distinct branches in the tree of lineages unite into a fundamentally novel, bipartite cell lineage. In other words, endosymbiosis involves the origin of novel taxa at higher level via the combinatorial union of cell lineages rather than via divergence—Darwin envisaged nothing of the sort. Even though the nuclear chromosomes of eukaryotes contain contributions from both host and endosymbiont lineages and even though the eukaryotes thus sit simultaneously on the eubacterial (mitochondrial) and archaebacterial (host) branches of the tree of cell lineages (*1*), most trees depicting the relatedness of cell lineages are drawn with bifurcating lines. Graphic depictions of life's history should explicitly reflect endosymbiotic processes and they should have some connection to the geological time scale, including major geochemical phases in terms of oxygen levels and the like.

Prokaryotes Early

The Earth is 4.5 billion years old and the ocean had condensed by ~4.4 Ga (billion years before present) (*11*). Life arose on Earth by ~3.8 Ga, because carbon isotope data provide evidence for biological CO_2-fixation in sedimentary rocks of that age (*12–14*). Recent criticism has been launched at some of the carbon isotope data from sediments at 3.8 Ga (*15*), but microprobe studies of such materials (*14*) are still accepted as indicating biological CO_2 fixation at 3.8 Ga (*15*). Microbial communities at hydrothermal vents existed by at least 3.2 Ga (*16*).

By about 2.7 Ga, prokaryotic communities were beginning to look very similar to many modern prokaryotic communities in terms of carbon and sulfur cycles (*12, 17–19*). This includes the isotopic trace of methanogenesis and methanotrophy by 2.7 Ga, suggesting that both methanogens (archaebacteria) and methanotrophs (α-proteobacteria, but possibly also including other groups (*20*)) were present at that time (*19*). Stromatolites, preserved microbial mats deposited by photosynthetic prokaryotes, were present as early as 3.5 Ga and have a more or less continuous record to the present (*21*).

Such evidence indicates that most of the biochemical pathways that drive modern prokaryotic carbon, sulfur and nitrogen cycles were in place by as early as 3.5 Ga, by 2.7 Ga at the latest (*12, 19*). Accordingly, it seems reasonable to assume that major lineages of eubacteria and archaebacteria were present and well-diversified by that time.

Oxygen Late and Surprising Sulfide

The Earth's early atmosphere contained either no O_2 at all or only very minor trace amounts. Today's O_2 stems from oxygenic photosynthesis in cyanobacteria and plastids (*22*). The time at which O_2 production in the oceans began—that is, the time of the origin of oxygenic photosynthesis—is uncertain. The abundance of ultra-light organic carbon bearing the isotope signature of methanotrophy (an oxygen-dependent pathway in eubacteria) and the study of microbial communities strongly indicates that oxygen was available at least as early as 2.7 Ga (*23*), consistent with evidence from cyanobacterial biomarkers at 2.7 Ga (*24*). Evidence from carbon cycles suggests that global oxygen production has been constant within an order of magnitude over the last 3.5 Ga (*12*). Overall, it seems safe to surmize that oxygen production in the oceans (hence the origin of cyanobacteria) occurred at least by 2.7 and possibly as early as 3.5 Ga. It also seems safe to assume that as soon as oxygen was available in the oceans, prokaryotes immediately discovered ways to utilize its power as an electron acceptor.

Various lines of geochemical evidence suggest that oxygen did not start accumulating in the atmosphere until about 2 billion years ago, at which point atmospheric O_2 rose sharply from $< 1\%$ of present atmospheric levels (PAL) to about 15% PAL during a small window of time from 2.2 to 2.1 Ga (*12, 25–28*). The sulfur isotope record and carbon deposition rates suggest that a second sharp rise in atmospheric O_2 approaching present levels occurred around ~0.6 Ga (*29*). But during the time from 2.2 to ~0.6 Ga, where atmospheric oxygen levels were about 15% PAL, deep ocean water was, according to newer findings, still anoxic and furthermore highly sulfidic (*30, 31*). That is, it contained no oxygen and high levels of sulfide as HS^-/H_2S. The evidence for this stems from stable sulfur isotope studies that reveal high activities of marine biological sulfate reduction—which produces sulfide—during that time (*31*).

Taken together, that evidence suggests (i) that cyanobacteria existed by at least 2.7 Ga, (ii) that there was little oxygen in the atmosphere or ocean before 2.2 Ga, (iii) that between

2.1 Ga and 0.6 Ga there was roughly 15% PAL O_2 in the atmosphere but none in deep ocean water, which was furthermore rich in sulfide, (iv) and that at $\sim$0.6 Ga O_2 levels in the atmosphere and deep ocean water approached present levels. That means that the eukaryotic lineage, which arose well before 0.6 Ga, underwent the brunt of its diversification in a largely anoxic and sulfidic world.

Eukaryotes: Younger Than Prokaryotes

There is no consensus among biologists concerning the position of the eukaryotes in the overall scheme of cell evolution (*1*). Current opinions on the origin/position of eukaryotes span a broad spectrum including the views (i) that eukaryotes arose first in evolution and that prokaryotes descend from them (*32*), (ii) that eukaryotes arose contemporaneously with eubacteria and archaebacteria and hence represent a primary line of descent of equal age and rank as the prokaryotes (*33*), (iii) that eukaryotes arose through a symbiotic event entailing an endosymbiotic origin of the nucleus (*34–37*), (iv) that eukaryotes arose without endosymbiosis (*38*), (v) that eukaryotes arose through a symbiotic event entailing a simultaneous endosymbiotic origin of the flagellum and the nucleus (*39*), in addition to other models summarized elsewhere (*40*). Here, a minority view on the place of eukaryotes in the overall scheme of things will be discussed, without devoting attention to the many alternative models, all of which have their virtues and to which the interested reader is referred.

When linking up prokaryotes and eukaryotes in a common picture of cell evolution, something always came prior and something came before that, etc., such that in order to have a complete picture (regardless of whether it is correct or not) one eventually has to start at the origin of life (but not dwell on it too long). A case can be marshalled for the view that life arose autotrophically in naturally forming compartments made of FeS precipitates at the bottom of the Hadean ocean (*41*). Under that view, the first cells were chemolithoautotrophs who satisfied their carbon needs through CO_2 alone and who satisfied their energy needs through redox reactions involving environmentally available inorganic donors and acceptors such as H_2, CO_2, CO, HS^-, and metal ions (*42*).

The view of 'autotrophic origins' was initially formulated on the basis of the concept that the reductive citric acid cycle was the first biochemical pathway and that the origin of metabolism involved pyrite (FeS_2) formation (*43*). However, some proponents of autotrophic origins now favour the view that the linear acetyl-CoA pathway of CO_2 fixation may have preceded all other CO_2 fixation pathways (*43, 44*). This is because the acetyl-CoA (or Wood-Ljungdahl) pathway is chemically quite simple, because it has very favourable thermodynamics towards CO_2 fixation, and because its catalysis is mediated largely by mineral components (FeS and FeNiS clusters) in a single bifunctional enzyme: carbon monoxide dehydrogenase/acetyl-CoA synthase (*43, 45*). In addition, acetyl-methylsulfide, an energy-rich thioester and analog of acetyl-CoA, can be synthesized from carbon monoxide and methylsulfide at $>4\%$ yields in the lab overnight in the presence of FeS and NiS without enzymes (*46*), implicating a role of reactions analogous to that catalyzed by acetyl-CoA synthase in primordial biochemistry.

Under the view of autotrophic origins, the heterotrophic lifestyle of microbes had to arise later than the autotrophic lifestyle, because without preexisting autotrophs to produce ample reduced organic compounds, heterotrophs cannot survive. All eukaryotes are ancestrally heterotrophs, they gain their energy through the oxidative breakdown of reduced carbon compounds (for example carbohydrates) that they obtain from the environment. Thus, under any scheme of cell evolution embracing autotrophic origins, eukaryotes have to postdate prokaryotes in origin (*42, 47*). But postdate by how much? If prokaryotes arose by at least 3.5 Ga, then what does the geological record say about the age of eukaryotes?

By about 1.5 Ga acritarchs become reasonably abundant, fossil unicellular organisms that are almost certainly eukaryotes (*48*) and probably algae by virtue of an easily preserved cell wall. By 1.2 Ga, very well-preserved multicellular red algae appear (*49*). Evidence of this type is accepted by most—but not by all (*38*)—as indicating that eukaryotes are at least 1.5 billion years old and that the diversification of the red algal lineage (which is not the most ancient lineage of algae) into multicellular forms occurred at least 1.2 billion years ago.

There have been reports of more ancient remains claimed to be eukaryotes, but they are often viewed with skepsis (*38, 42*). For example the filamentous fossil *Grypania* occurs at 2.1 Ga (*50*), but it could just as easily be a filamentous prokaryote as a filamentous eukaryote, because the cellular structure of the material is not preserved. This is in contrast to *Bangiomorpha* at 1.2 Ga (*49*), the large-celled, truly multicellular structure of which is strikingly preserved. Steranes were recently found in 2.7 Ga sediments and it was claimed that these biomarkers provide evidence for the existence of eukaryotes at that time (*51*). But several groups of prokaryotes including methanotrophic α-proteobacteria (*52*), myxobacteria (*53*), and cyanobacteria (*54*) make the same kinds of compounds (for example cholesterol) claimed to be eukaryote-specific, such that the sterane evidence appears to document biochemically diverse prokaryotes, rather than the existence of eukaryotes.

In sum, there is convincing evidence that eukaryotes (probably algae) were in existence by 1.5 Ga, and that multicellular red algae existed by 1.2 Ga. Since the origin of algae entails the origin of plastids from cyanobacteria, and since the host that acquired plastids possessed mitochondria, the origin of mitochondria should be sought well before 1.2 Ga and somewhere before 1.5 Ga.

Eukaryotes and Mitochondria: Origins in Anaerobic Times

The evidence summarized above indicates that deep ocean water was anoxic and sulfidic up until about 0.6 Ga and and

that the origin of mitochondria dates back to at least 1.5 Ga. Therefore, mitochondria must have arisen in a global setting where marine oxygen levels were extremely low and sulfide levels were high. Furthermore, the first ~1 billion years (at least) of eukaryote diversification occurred in a marine environment marked by low oxygen, widespread anoxia and high sulfide. It is therefore not surprising that many eukaryotes still thrive today in anaerobic environments (*55*), some of which, such as marine sediments, are also sulfide-rich (*56*). On the basis of their ATP-synthesizing pathways, modern anaerobic eukaryotes can be divided into three unnatural groups: those that possess anaerobically functioning mitochondria (*57*), the so-called Type II eukaryotes which synthesize ATP in hydrogenosomes, and the so-called Type I eukaryotes which possess neither typical mitochondria nor hydrogenosomes and synthesize all of their ATP in the cytosol (*58–60*).

It was once thought that parasitic eukaryotes such as the microsporidians (*61*) or Type I eukaryotes such as the diplomonad *Giardia lamblia* (*62*), which gain ATP without the help of mitochondria or hydrogenosomes, might be the most ancient among contemporary groups and that they might have never possessed a mitochondrion at all. But starting about 1995, numerous studies revealed eukaryotes that lack mitochondria to have possessed a mitochondrion in their evolutionary past (reviewed in *63–65*), or to even still possess a long-overlooked, highly reduced remnant mitochondrion with no apparent function in ATP synthesis called a mitosome (*66, 67*). Accordingly, it seems that mitochondria are as ancient as eukaryotes themselves and that the loss of mitochondria has occurred many times independently in various eukaryotic lineages (*10, 60, 63–65*).

Hydrogenosomes—the double-membrane bounded, H_2-producing and ATP-producing organelles of various anaerobic eukaryotes (*10, 40, 55, 58, 68, 69*)—figure prominently in understanding early eukaryotic history. Hydrogenosomes are specifically suited to eukaryotic life in anaerobic environments and they harbour many O_2-sensitive enzymes such as pyruvate:ferredoxin oxidoreductase, [Fe]-hydrogenase and pyruvate-formate lyase (*10, 70–73*). Hydrogenosomes occur in at least four highly disparate groups of eukaryotes—trichomonads, ciliates, amoeboflagellates, and chytridiomycete fungi (*59*)—and are now known to be anaerobic forms of mitochondria (*10, 40, 59, 69*). The evolutionary significance of hydrogenosomes is evident: they bridge the gap between ATP synthesis in aerobic and anaerobic eukaryotes, because they contain enzymes common both to mitochondria and to cytosolic ATP synthesis in Type I eukaryotes (*57, 60*). Hydrogenosomes forge a biochemical link between the largely anaerobic ancient phases of eukaryotic history and the more recent past—the last 600 million years—during which time aerobic niches have become more widespread and anaerobic environments (for example sediments) have become more restricted. A model that specifically accounts for the common origin of mitochondria and hydrogenosomes from a single (facultatively anaerobic) eubacterial ancestor, that specifically predicts no eukaryote to be primarily amitochondriate, that specifically accounts for the origin of heterotrophy in eukaryotes, and that specifically accounts for anaerobic mitochondria (*57*) has been presented elsewhere (*60*), also in sufficient detail as to account for an endogenous origin of the nucleus subsequent to the origin of mitochondria (*42*). Alternative models for the origins of eukaryotes mentioned above are designed to account for other things.

Eukaryote Phylogeny: a Tree Turned Upside Down

Traditional views of eukaryote phylogeny are based in the classical rRNA tree, which depicts various anaerobic and amitochondriate eukaryotes branching deeply and the animals, fungi and plants emerging as the latest lineages of eukaryotic evolution—but that view is now outdated. Newer investigations of many genes (rather than just a single gene) are uncovering evidence for the existence of a relatively small number of major eukaryotic lineages. These include well-recognized groups such as plants with primary plastids, animals, and fungi, but also including new and surprising groups, sometimes with unfamiliar names such as excavates, amoebozoa, opisthokonts, chromalveolates and the like (*38, 63, 64, 74–78*).

Those are exciting developments. But perhaps more important in the overall scheme of things than the sorting out of 'who belongs where' in terms of groupings is the position of the root in the eukaryotic tree, that is, the question of which lineages of eukaryotes might be the oldest. Because of the way that phylogeny algorithms work, the rRNA tree seems to have consistently produced a severe artefact with regard to the placement of the root. This is mainly because when eukaryote rRNA sequences are linked up to prokaryote rRNA sequences in the same tree, the outgroup (prokaryote) branch will tend to fall among the longest eukaryote branches, regardless of whether those long-branched sequences are the most ancient or whether they are simply the most different (*63, 64*).

New evidence from the study of a particular gene fusion involving dihydrofolate reductase and thymidylate kinase that is found only among some eukaryotes, has strongly suggested that the root in the eukaryotic tree lies on or very near the branch that separates animals and fungi from all other eukaryotes (*75*). This rooting is highly compatible with the new handful of perhaps six eukaryotic 'supergroups' that are currently emerging from multi-gene phylogenies (*76*).

Fungi First Would Make Sense

From the standpoint of energy metabolism, and beyond the strength of the gene fusion data itself, the rooting of Stechmann and Cavalier-Smith (*75*), or 'opisthokont root' is very attractive, because it would implicate the fungi as one of the most ancient eukaryotic lineages (opisthokonts is a term

coined by Cavalier-Smith to designate the group comprising animals and fungi on the basis of locomotion in unicellular stages). Compared to prokaryotes, eukaryotes have only a miniscule diversity of core energy metabolic pathways for sustained ATP-synthesis. But on the basis of available data, it seems that fungi have the broadest energy metabolic (physiological) diversity of any eukaryotic group. The fungi encompass many species with typical aerobic mitochondria, species with anaerobic mitochondria that can perform nitrite respiration (*79*), species with hydrogenosomes (*72, 73, 80, 81*), species that can perform a hitherto unique feat among eukaryotes called ammonia fermentation (*82*), groups with extremely reduced mitochondria (*67*), and groups that perform methylotrophy, that is, they can live from methanol as their sole carbon and energy source (*83*), something no other eukaryotes to the authors' knowledge can.

Furthermore, the fungi as a group are osmotrophs, not phagotrophs. They take up their nourishment with the help of membrane-localized importers, just like phagotrophs do, but they do not phagocytose large particles as food vacuoles. The digestion enzymes that phagocytotic eukaryotes excrete into food vacuoles, fungi excrete into their environment. The importers that phagocytotic eukaryotes use to import digest from food vacuoles reside on the plasma membrane in fungi. It is conceivable that the fungi as a group could have diverged from the main stem of eukaryotic evolution before proper phagocytosis had evolved. That notion is not likely to become popular, because most biologists still tend firmly towards the view that phagocytosis was a prerequisite for the origin of mitochondria, a view that is however founded more in tradition than in evidence. Examples of prokaryotic endosymbionts that live within prokaryotic hosts incapable of proper phagocytosis are known (*84*). By analogy, the origin of mitochondria need not have absolutely demanded phagocytosis of its host.

Regardless of how eukaryotes arose and which group is the most ancient, available evidence suggests that both the presence of a nucleus and the presence of a mitochondrial endosymbiont, which in some cases may be highly reduced or possibly lost altogether, are defining features of eukaryotes (*38, 85*). Furthermore, available evidence indicates that mitochondria arose only once in evolution (*74, 86*). In view of the hefty number of unicellular organisms that have ever lived, the origin of mitochondria was an unspeakably rare event.

Origins of Plastids, Primary and Secondary

Chloroplasts arose from cyanobacteria through primary endosymbiosis and available evidence indicates that their origin, too, was a singular event in evolution (*87–89*), followed by a still uncertain number (between two and seven) of secondary endosymbiotic events in which a eukaryotic host engulfed a eukaryotic alga (*89–95*). Molecular phylogenetic studies have yet to link plastids robustly with any particular group of contemporary cyanobacteria, although genome-based study suggested that the heterocyst-forming cyanobacterium *Nostoc* might share more overall similarity to the ancestor of plastids than the unicellular cyanobacterium *Synechocystis* PCC 6803 or the prochlorophyte *Prochlorococcus* (*96*). But with only three cyanobacterial genomes in that comparison, there is unlimited room for additional taxon sampling among cyanobacterial genomes.

Contemporary chloroplast genomes encode between 60–200 proteins in various photosynthetic lineages and have thus undergone a process of severe genome reduction during the course of endosymbiosis, because contemporary cyanobacteria encode several thousand proteins. But plastids contain roughly just as many proteins as their free-living cyanobacterial cousins, recent estimates suggesting that about 3000 proteins in higher plants are targeted to plastids (*97*). Many gene transfers to the nucleus have occurred during plastid evolution. Current estimates indicate that about 4500 genes in the *Arabidopsis* genome come from cyanobacteria in the form of transfers from the ancestral plastid genome (*96*). This amounts to about 18% of the nuclear-encoded proteins in the *Arabidopsis* genome—a significant contribution from the organelle to the nuclear genetic makeup of higher plants. Genes are also transferred from plastids to the nucleus during the course of secondary endosymbiosis (*98*). Dramatic new findings have directly demonstrated the process of gene transfer from plastids to the nucleus in greenhouse crosses with transgenic tobacco (*99*), revealing that the mechanism of gene transfer from organelles to the nucleus involves direct transfer and chromosomal integration of bulk DNA rather than cDNA intermediates as was once thought.

The number of different secondary symbiotic events that occurred during plastid evolution is an important but yet unresolved issue. At the focus of much debate is the concept of the chromalveolates (*92, 100, 101*), a group that unites seemingly disparate algal lineages by virtue of their supposed common ancestry from one and the same secondary endosymbiotic event involving a red algal plastid. Among the chromalveolates are the apicomplexans, whose plastids clearly appear to stem from red algae on the basis of plastid genome data (*87, 89*), though other evidence hints towards a green algal ancestry (*94, 102*), which would not mesh with the chromalveolates being a natural group. Whether or not the chromalveolates hold will have a large influence on the number of secondary endosymbioses that we need to assume to have occurred in protist evolution.

The fossil evidence mentioned in earlier passages suggests that the primary symbiosis which gave rise to algae (hence plastids) occured well before 1.2 Ga, perhaps some 1.5 billion years ago. An interesting aspect of the opisthokont-rooted eukaryotic tree (*75*) is that it does not demand any prolonged phase of extensive eukaryotic lineage-diversification between the origins of mitochondria and the origins of plastids. The origins of these organelles could conceivably have followed

each other in relatively rapid succession. Accordingly, the origins of mitochondria predate the origins of plastids, but possibly not by much, such that mitochondria (and eukaryotes) might be about 2 billion years old, which is compatible with molecular clock estimates (*2*).

Low Oxygen and High Sulfide up to 600 Million Years Ago: the Major Consequences

In a recent review, Anbar and Knoll (*31*) pointed out one possible consequence that the evidence for anoxic and sulfidic oceans would have had upon eukaryotic diversity. Their case was that high sulfide up until ~0.6 Ga would have kept marine concentrations of certain metal ions such as iron and molybdenum very low. This, in turn (so goes the argument), could ultimately limit algal diversification by hampering prokaryotic nitrogen fixation, which requires these metals in order to operate. By this reasoning, one could account for low levels of eukaryotic microfossil diversity observed prior to ~1 Ga. Although the argument of Anbar and Knoll (*31*) is not fundamentally flawed, it probably misses the main point.

It is true that iron can limit cyanobacterial biomass in the oceans (*103*). But from the standpoint of eukaryotic microbial physiology, the main consequence of anoxic and sulfidic oceans would not have been the gradual problem of dealing with low nitrogen availability (slow starvation). Rather, eukaryotes would have seen themselves faced with the immediate and (for aerobes) life-threatening problem of dealing with recurrent or permanent anoxia and sulfide (asphyxia and poisoning) on a daily basis for over half a billion years. The major consequences from this simple consideration are threefold.

First, in that anoxic world, anaerobic energy metabolism in mitochondrion-containing cells would have been a prerequisite for survival, an absolute must, a *conditio sine qua non*. The consequence is that ancestral eukaryotes must have possessed enzymes for sustained ATP synthesis under anoxic conditions. Accordingly, it would hardly be surprising to find the trace of that ancestral anaerobic energy metabolism in mitochondrion- or hydrogenosome-bearing cells today, particularly in such lineages as inhabit anaerobic niches. Indeed, such anaerobic biochemistry is abundant among eukaryotes (*55–60*). Our argument (*40*, *57*, *60*) has been—and remains—that the fabric of that anaerobic biochemistry almost certainly represents a holdover from the ancestral eukaryotic state (facultatively anaerobic; in this specifc case: possessing a respiratory chain and capable of anaerobic fermentations). Importantly, the presence of anaerobic energy metabolism in ancestral mitochondria in no way excludes the presence of a respiratory chain in addition. We are often misunderstood on this point. The hydrogen hypothesis posits that the initial symbiosis between the ancestor of mitochondria and its host was mediated by anaerobic syntrophy based upon the ability of the symbiont to produce molecular hydrogen under anaerobic conditions, but (obviously) also suggests that the ancestral mitochondrion was also able to respire oxygen (*60*). The inference from that premise as it regards the symbiont is that the common ancestor of mitochondria and hydrogenosomes was simply a facultatively anaerobic α-proteobacterium, one with a heterotrophic physiology perhaps similar to modern day *Rhodobacter* or countless other photosynthetic and nonphotosynthetic representatives of the group (*60, 70*). There is nothing unusual about being facultatively anaerobic, *E. coli* is a facultative anaerobe and produces hydrogen under anaerobic conditions (*55*) (albeit via a different pathway than hydrogenosomes use). Accordingly, hydrogenosomes would have preserved their ancient anaerobic biochemistry and would have secondarily lost the ability to respire in many lineages. Conversely, typical mitochondria would have preserved their ancient aerobic biochemistry (respiration) and at ~0.6 Ga with the advent of fully aerobic environments would have secondarily lost fermentative pathways in many lineages. By similar reasoning, eukaryotes with primary plastids have been producing their own oxygen locally for over a billion years, and many such lineages may therefore have lost much of their (ancestrally existing) anaerobic energy metabolism early on. Based upon the newer geochemical evidence (*29–31*), the conclusion seems inescapable that eukaryotes arose and spent the brunt of their evolutionary youth in an anoxic world. Hence the still widely held view that hydrogenosomes are merely biochemically modified mitochondria, having secondarily tacked on anaerobic enzymes to an implicitly (*67*) or explicitly (*104*) strictly aerobic ancestral state is inconsistent with the newer geochemical data and seems very difficult to uphold.

Second, the geochemical evidence for largely anoxic oceans before ~0.6 Ga very strongly suggests that up until that time, the anaerobic biochemistry in hydrogenosomes must have been much more widespread among eukaryotes than it is today. This would significantly help to explain why the H_2-producing fermentations in hydrogenosomes of such distantly related groups as the cytridiomycete fungi, the ciliates, and the trichomonads are so similar in overall design (*59*) and are performed with enzymes that were present in the common ancestor of those lineages (*10, 71*). It also significantly helps to explain the widespread distribution of anaerobic mitochondria, even among metazoan lineages (*57*). Despite the fact that hydrogenosomes have been known for 30 years, classical endosymbiotic theory has never been able to accomodate them (*39*), mainly because it is designed to account for aerobic mitochondria only (*104*). Under the view that mitochondria had aerobic origins, the biochemical unity of hydrogenosomes from different eukaryotic lineages (*59*) would be altogether inexplicable—under the view that mitochondria had anaerobic origins, hydrogenosomes are the key to the eukaryotic biochemical past.

Third, during the entire period from the time of their origins up until 0.6 Ga, eukaryotes had to deal with very high levels of sulfide, which is a potent toxin. Many contemporary

marine invertebrates (metazoans) still have to deal with very high sulfide concentrations, particularly those that live in coastal sediments. Such organisms use a mitochondrial enzyme, sulfide:quinone oxidoreductase to oxidize sulfide to the less toxic product thiosulfate, whereby the electrons from sulfide oxidation are fed into the electron transport chain to generate chemiosmotic potential for mitochondrial ATP synthesis (*105, 106*), just as it occurs in many sulfide-utilizing eubacteria today (*107, 108*). That biochemical trace of our sulfidic past is even preserved up into the vertebrate lineage, because chicken mitochondria can also oxidize sulfide to drive ATP synthesis (*109*). The gene for mitochondrial sulfide:quinone oxidoreductase has been identified in fungi (*110*) and gene phylogenies indicate a single origin for the eukaryotic enzyme, suggesting that this gene was indeed present in the respiratory chain of the ancestral mitochondrion (*111*).

All things considered, the ability of modern mitochondria to deal with anoxia and sulfide are most easily understood as biochemical relics from the anoxic and sulfidic beginnings of the eukaryotic lineage.

A Diagram and its Intended Meaning

The main considerations of this paper are summarized as a schematic diagram of cell lineage history with a rough time scale in Fig. 1. Some specialists will object to several aspects of the figure, so a few things need explaining.

We have depicted the host lineage of eukaryotes as emerging from within the archaebacteria, rather than as a sister to the archaebacteria as in many gene trees. This is primarily founded in the consideration that eukaryotes have no chemoautotrophic forms and therefore must be younger than either archaebacteria or eubacteria (*42*, *47*, *60*) consistent with evidence from the geological record outlined in earlier sections. Conversely, archaebacteria must be older than eukaryotes, and we can envision no rational evolutionary reason why archaebacteria should have failed to diversify into lineages during their roughly two billion years of existence prior to the origin of eukaryotes—there certainly was an ample environmental supply of the compounds that diverse archaebacterial lineages need to survive (*113*) during that time. In addition, some archaebacteria possess histones (*114*) whereas others do not, which we would take as evidence linking eukaryotes closer to some lineages of archaebacteria than to others. Furthermore, gene trees that show archaebacteria and eukaryotes as sisters entail eubacterial outgroups and ancient duplicated genes (*115*). Much ado has been made about the long branch attraction artefact in phylogeny, which causes the most different sequences to branch deeply in a tree, regardless of whether they are the most ancient or not, and the sisterhood of archaebacteria and the eukaryotic host lineage is not immune to this phenomenon either (*116*).

We have also labelled a group as 'ancestrally photosynthetic others', which is a category (not phylogenetic assemblage) intended to include Glaucocystophytes, currently thought to be the earliest-branching lineage with primary plastids. But this category might ultimately turn out to encompass other groups, because it is still somewhat uncertain whether some lineages of eukaryotes possessed a primary plastid that they secondarily lost (*117*). However, secondary loss of secondary plastids appears to be much more common than secondary loss of primary plastids (*89*).

Regarding our speculations in this paper concerning the possible basal position of fungi among eukaryotes (drawn unresolved in the figure), it should be mentioned that evidence from the distribution of indels in several genes suggested animals and fungi to have a specific sister group relationship (*118*). That grouping is now widely accepted and it would be difficult to reconcile with a genuinely basal position of fungi unless one allows for a couple of gene duplications and differential loss. But it also should be mentioned that indels themselves can result from parallel evolution in independent lineages (*119*). In other words, indels provide readily visible evidence for phylogeny, but evidence from indels is sometimes inconsistent (contains conflicting signals) and indels are by no means infallible as phylogenetic markers (*119*). The notion that fungi might be the most primitive and earliest-branching eukaryotes—as Cavalier-Smith once argued, albeit at a time when the symbiotic origins of mitochondria was not accepted by all (*120*)—is attractive from a physiological standpoint and in the context of models for the origin of mitochondria that invoke an archaebacterial host (*42, 60*).

The view that osmotrophy had to precede phagotrophy in eukaryotic evolution is compelling because without importers, food vacuoles are useless. That all fungi are osmotrophs and that none are phagotrophs could mean that their common ancestor was either primitively or secondarily non-phagotrophic. This leads to the subtle question of how eukaryotes became osmotrophs in the first place. Osmotrophy requires substrate importers at the plasma membrane and cytosolic carbon metabolism suited to the heterotrophic lifestyle (ATP synthesis through the oxidation of reduced organic compounds). In yeast, heterotrophy entails eubacterial importers in the plasma membrane, eubacterial carbon metabolism in the cytosol, and a eubacterial organelle (*121, 122*). This observation at its level of resolution is generally compatible with the predictions that stem directly from three current models for the origin of eukaryotes: (i) The host that acquired the mitochondrion was a member of the actinobacteria (a group of Gram positive eubacteria that includes actinomycetes) that had become a phagotrophic eukaryote (*38*). (ii) Eukaryotes arose through symbiosis in which a methanogen became the nucleus in a δ-proteobacterial host (*36*). (iii) The host that acquired the mitochondrion was an autotrophic archaebacterium that acquired through endosymbiotic gene transfer the prexisting heterotrophic lifestyle of its α-proteobacterial symbiont (*60*). Notwithstanding LGT (*1*), discriminating genome analyses are needed to see if eukaryotic genes, particularly those involved in osmotrophy, share more

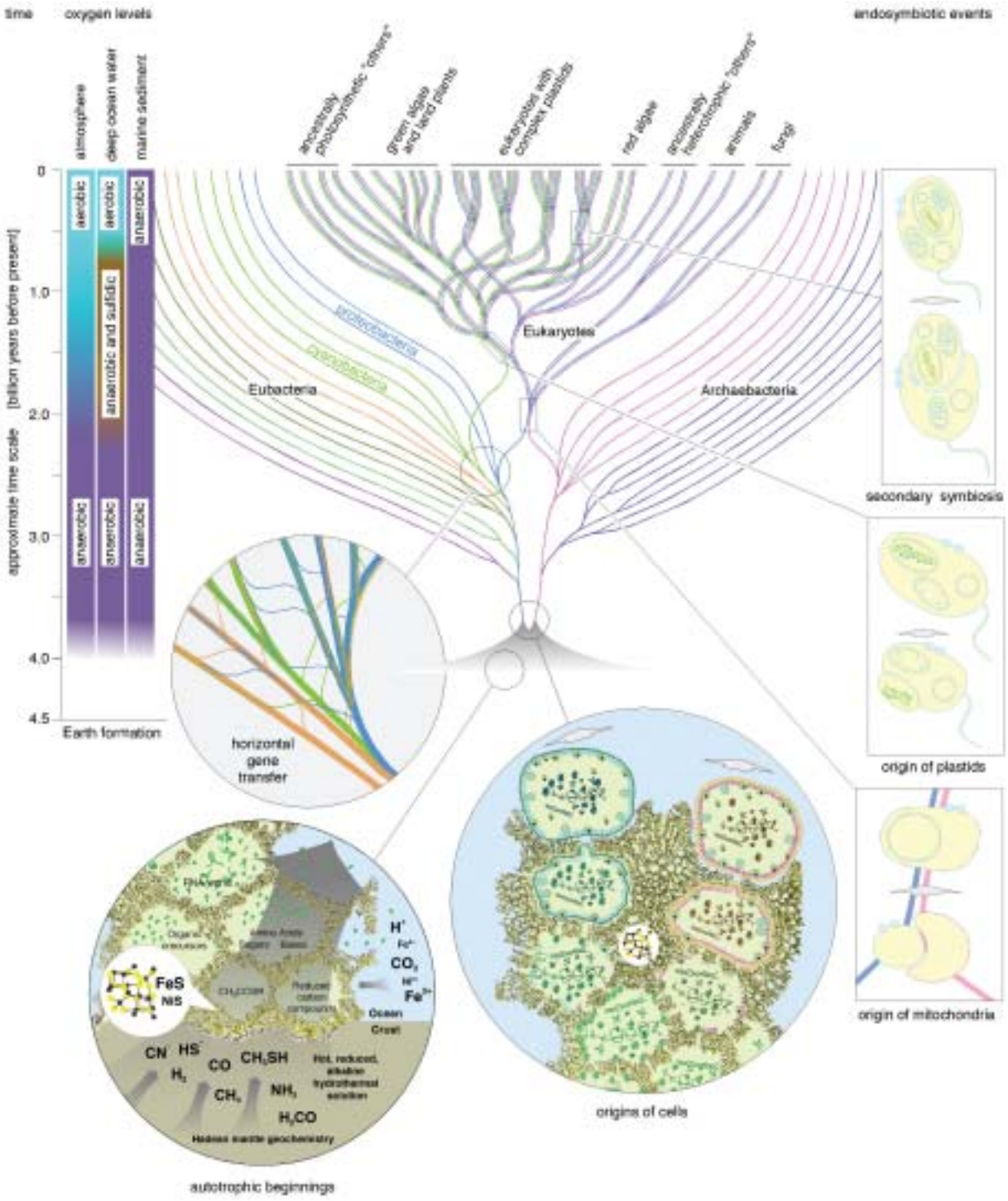

Figure 1. A graphic summary of important events in the history of unicellular life and some justifiable guesswork as to their approximate times. Oxygen levels in the atmosphere and deep ocean water are summarized from (*31*). Oxygen levels in marine sediment are summarized from (*55*) and (*56*). Sketches for autotrophic beginnings and origins of cells are redrawn from (*41*). The basic shape of the tree is redrawn from (*112*) with the rooting as indicated in (*75*). Times of key events are mentioned in the text. Little blue dots in the plasma membrane of cells in the right panel symbolize eubacterial importers.

similarity with their homologues distributed among actinobacteria (*38*), δ-proteobacteria (*36*), or α-proteobacteria (*60*).

CONCLUSION

Lateral gene transfer has dashed the hopes of quick success at fully uncovering life's history with genomes. But it has opened up new ways of looking into the past with the binoculars of sequence comparisons (*1–7*). Geologists are telling us that the Earth's oceans were largely anoxic and highly sulfidic for much longer that was previously thought (*28–31*). Phylogeneticists are telling us that the classical rRNA tree has eukaryotic evolution completely upside-down (*75–78*). From the standpoint of physiology, fungi look promising as genuine early-branchers. A better understanding of our unicellular ancestors and the environments in which they lived 1.5 billion years ago can help us make sense of the genomes that we sequence today.

ACKNOWLEDGEMENTS

We thank the Deutsche Forschungsgemeinschaft for financial support.

REFERENCES

1. Brown, J. R. (2003) Ancient horizontal gene transfer. *Nat. Rev. Genet.* **4**, 121–132.
2. Feng, D.-F., Cho, G., and Doolittle, R. F. (1997) Determining divergence times with a protein clock: update and reevaluation. *Proc. Natl. Acad. Sci. USA* **94**, 13028–13033.
3. Doolittle, W. F., Boucher, Y., Nesbo, C. L., Douady, C. J., Andersson, J. O., and Roger, A. J. (2003) How big is the iceberg of which organellar genes in nuclear genomes are but the tip? *Phil. Trans. R. Soc. Lond. B. Biol. Sci.* **358**, 39–58.
4. Ochman, H., Lawrence, J. G., and Groisman, E. S. (2000) Lateral gene transfer and the nature of bacterial innovation. *Nature* **405**, 299–304.
5. Eisen, J. (2000) Horizontal gene transfer among microbial genomes: new insights from complete genome analysis. *Curr. Opin. Genet. Dev.* **10**, 606–611.
6. Katz, L. A. (2002) Lateral gene transfers and the evolution of eukaryotes: theories and data. *Int. J. Syst. Evol. Micr.* **52**, 1893–1900.
7. Deppenheimer, U., Johann, A., Hartsch, T., Merkl, R., Schmitz, R. A., Martinez-Arias, R., Henne, A., Weizer, A., Baumer, S., and Jacobi, C., (2002) The genome of *Methanosarcina mazei*: Evidence for lateral gene transfer between bacteria and archaea. *J. Mol. Microbiol. Biotechnol.* **4**, 453–461.
8. Penny, D., McComish, B. J., Charleston, M. A., and Hendy, M. D. (2001) Mathematical elegance with biochemical realism: The covarion model of molecular evolution. *J. Mol. Evol.* **53**, 711–723.
9. Salzberg, S. L., White, O., Peterson, J., and Eisen, J. A. (2001) Microbial genes in the human genome: lateral transfer or gene loss? *Science* **292**, 1903–1906.
10. Embley, T. M., van der Giezen, M., Horner, D. S., Dyal, P. L., and Foster, P. (2003) Hydrogenosomes and mitochondria: phenotypic variants of the same fundamental organelle. *Phil. Trans. Roy Soc. Lond. B.* **358**, 191–203.
11. Wilde, S. A., Valley, J. W., Peck, W. H., and Graham, C. M. (2001) Evidence from detrital zircons for the existence of continental crust and oceans on the Earth 4.4 Gyr ago. *Nature* **409**, 175–178.
12. Nisbet, E. G. and Sleep, N. H. (2001) The habitat and nature of early life. *Nature* **409**, 1083–1091.
13. Rosing, M. T. (1999) 13C-depleted carbon microparticles in > 3700-Ma sea-floor sedimentary rocks from west Greenland. *Science* **283**, 674–676.
14. Ueno, Y., Yurimoto, H., Yoshioka, H., Komiya, T., and Maruyama, S. (2002) Ion microprobe analysis of graphite from ca. 3.8 Ga metasediments, Isua crustal belt, West Greenland: Relationship between metamorphism and carbon isotopic composition. *Geochimica Cosmochimica Acta* **66**, 1257–1268.
15. van Zuilen, M. A., Lepland, A., and Arrhenius, G. (2002) Reassessing the evidence for the earliest traces of life. *Nature* **418**, 627–630.
16. Rasmussen, B. (2000) Filamentous microfossils in a 3,235-million-year-old volcanogenic massive sulphide deposit. *Nature* **405**, 676–679.
17. Nisbet, E. G., and Fowler, C. M. R. (1999) Archaean metabolic evolution of microbial mats. *Proc. Roy. Soc. Lond. B.* **266**, 2375–2382.
18. Nisbet, E. (2000) The realms of Archaean life. *Nature* **405**, 625–626.
19. Grassineau, N. V., Nisbet, E. G., Bickle, M. J., Fowler, C. M. R., Lowry, D., Mattey, D. P., Abell, P., and Martin, A. (2001) Antiquity of the biological sulphur cycle: evidence from sulphur and carbon isotopes in 2700 million-year-old rocks of the Belingwe Belt, Zimbabwe. *Proc. Roy. Soc. Lond. B.* **268**, 113–119.
20. Michaelis, W., Seifert, R., Nauhaus, K., Treude, T., Thiel, V., Blumenberg, M., Knittel, K., Gieseke, A., Peterknecht, K., and Pape, T., *et al.* (2002) Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. *Science* **297**, 1013–1015.
21. Walter, M. R. (1994) Stromatolites: The main source of information on the evolution of the early benthos. In *Earth's Earliest Biosphere: Its Origin and Evolution* (Schopf, J. W, ed.). pp. 270–286, Princeton University Press, NJ.
22. Kasting, J. F., and Seifert, J. L. (2002) Life and the evolution of Earth's atmosphere. *Science* **296**, 1066–1068.
23. Hayes, J. M. (1994) Global methanotrophy at the Archean-Proterozoic transition. In *Early Life on Earth* (Bengston, S. ed.). pp. 220–236. Columbia Univ. Press, New York.
24. Canfield, D. E. (1999) A breath of fresh air. *Nature* **400**, 503–504.
25. Wiechert, U. H. (2002) Earth's early atmosphere. *Science* **289**, 2341–2341.
26. Holland, H. D. (1999) When did the Earth's atmosphere become oxic? *The Geochemical News* **100**, 20–22.
27. Holland, H. D., and Beukes, N. (1990) A paleoweathering profile from Griqualand West, South Africa: Evidence for a dramatic rise in atmospheric oxygen between 2.2 and 1.9 BYBP. *Am. J. Sci.* **290-A**, 1–34.
28. Karhu, J. A., and Holland, H. D. (1996) Carbon isotopes and the rise of atmospheric oxygen. *Geology* **24**, 867–870.
29. Canfield, D. E., and Teske, A. (1996) Late Proterozoic rise in atmospheric oxygen concentration inferred from phylogenetic and sulphur-isotope studies. *Nature* **382**, 127–132.
30. Canfield, D. E. (1998) A new model for Proterozoic ocean chemistry. *Nature* **396**, 450–453.
31. Anbar, A. D., and Knoll, A. H. (2002) Proterozoic ocean chemistry and evolution: A bioinorganic bridge. *Science* **297**, 1137–1142.
32. Forterre, P., and Philippe, H. (1999) Where is the root of the universal tree of life? *BioEssays* **21**, 871–879.
33. Woese, C. R. (2002) On the evolution of cells. *Proc. Natl. Acad. Sci. USA* **99**, 8742–8747.

34. Lake, J. A., and Rivera, M. C. (1994) Was the nucleus the first endosymbiont? *Proc. Natl. Acad. Sci. USA* **91**, 2880–2881.
35. Gupta, R. S. (1998) Protein phylogenies and signature sequences: A reappraisal of evolutionary relationships among archaebacteria, eubacteria, and eukaryotes. *Microbiol. Mol. Biol. Rev.* **62**, 1435–1491.
36. Moreira, D., and Lopez-Garcia, P. (1998) Symbiosis between methanogenic archaea and δ-proteobacteria as the origin of eukaryotes: the syntrophic hypothesis. *J. Mol. Evol.* **47**, 517–530.
37. Wächtershäuser, G. (2003) From pre-cells to Eukarya—a tale of two lipids. *Mol. Microbiol.* **47**, 13–22.
38. Cavalier-Smith, T. (2002) The phagotrophic origin of eukaryotes and phylogenetic classification of Protozoa. *Int. J. Syst. Evol. Microbiol.* **52**, 297–354.
39. Margulis, L., Dolan, M. F., and Guerrero, R. (2000) The chimeric eukaryote: Origin of the nucleus from the karyomastigont in amitochondriate protists. *Proc. Natl. Acad. Sci. USA* **97**, 6954–6959.
40. Martin, W., Hoffmeister, M., Rotte, C., and Henze, K. (2001) An overview of endosymbiotic models for the origins of eukaryotes, their ATP-producing organelles (mitochondria and hydrogenosomes), and their heterotrophic lifestyle. *Biol. Chem.* **382**, 1521–1539.
41. Russell, M. J., and Hall, A. J. (1997) The emergence of life from iron monosulphide bubbles at a submarine hydrothermal redox and pH front. *J. Geol. Soc. Lond.* **154**, 377–402.
42. Martin, W., and Russell, M. (2003) On the origins of cells: a hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells. *Phil. Trans. Roy. Soc. Lond. B.* **358**, 59–85.
43. Wächtershäuser, G. (1990) Evolution of the first metabolic cycles. *Proc. Natl. Acad. Sci. USA* **87**, 200–204.
44. Peretó, J. G., Velasco, A. M., Becerra, A., and Lazcano, A. (1999) Comparative biochemistry of CO_2 fixation and the evolution of autotrophy. *Internatl. Microbiol.* **2**, 3–10.
45. Lindahl, P. A. (2002) The Ni-containing carbon monoxide dehydrogenase family: light at the end of the tunnel? *Biochemistry* **41**, 2097–2105.
46. Huber, C., and Wächtershäuser, G. (1997) Activated acetic acid by carbon fixation on (Fe,Ni)S under primordial conditions. *Science* **276**, 245–247.
47. Kandler, O. (1994) The early diversification of life. In *Early Life on Earth* (Bengston, S. ed.) pp. 152–160, Columbia Univ. Press, New York.
48. Javaux, E. J., Knoll, A. H., and Walter, M. R. (2001) Morphological and ecological complexity in early eukaryotic ecosystems. *Nature* **412**, 66–69.
49. Butterfield, N. J. (2000) *Bangiomorpha pubescens* n. gen., n. sp.: implications for the evolution of sex, multicellularity, and the Mesoproterozoic/Neoproterozoic radiation of eukaryotes. *Paleobiology* **263**, 386–404.
50. Han, T. M., and Runnegar, B. (1992) Megascopic eukaryotic algae from the 2.1 billion-year-old Neeguanee iron formation, Michigan. *Science* **257**, 232–235.
51. Brocks, J. J., Logan, G. A., Buick, R., and Summons, R. E. (1999) Archean molecular fossils and the early rise of eukaryotes. *Science* **285**, 1033–1036.
52. Schouten, S., Bowman, J. P., Rijpstra, W. I., and Sinninghe Damste, J. S. (2000) Sterols in a psychrophilic methanotroph, *Methylosphaera hansonii*. *FEMS Microbiol. Lett.* **186**, 193–195.
53. Kohl, W., Gloe, A., and Reichenbach, H. (1983) Steroids from the myxobacterium *Nannocystis exedens*. *J. Gen. Microbiol.* **129**, 1629–1635.
54. Hai, T., Schneider, B., Schmidt, J., and Adam, G. (1996) Sterols and triterpenoids from the cyanobacterium *Anabaena hallensis*. *Phytochemistry* **41**, 1083–1084.
55. Fenchel, T., and Finlay, B. J. (1995) *Ecology and Evolution in Anoxic Worlds*, Oxford University Press, Oxford, New York, Tokyo.
56. Grieshaber, M. K., and Völkel, S. (1998) Animal adaptations for tolerance and exploitation of poisonous sulfide. *Ann. Rev. Physiol.* **60**, 30–53.
57. Tielens, A. G. M., Rotte, C., van Hellemond, J., and Martin, W. (2002) Mitochondria as we don't know them. *Trends Biochem. Sci.* **27**, 564–572.
58. Müller, M. (1993) The hydrogenosome. *J. Gen. Microbiol.* **139**, 2879–2889.
59. Müller, M. (1998) Enzymes and compartmentation of core energy metabolism of anaerobic protests–a special case in eukaryotic evolution? In *Evolutionary Relationships Among Protozoa* (Coombs, G. H., Vickerman, K., Sleigh, M. A., and Warren, A. eds.) pp. 109–131, Kluwer, Dordrecht.
60. Martin, W., and Müller, M. (1998) The hydrogen hypothesis for the first eukaryote. *Nature* **392**, 37–41.
61. Vossbrinck, C. R., Maddox, J. V., Friedman, S., Debrunner-Vossbrinck, B. A., and Woese, C. R. (1987) Ribosomal RNA sequence suggests microsporidia are extremely ancient eukaryotes. *Nature* **326**, 411–414.
62. Sogin, M., Gunderson, J., Elwood, H., Alonso, R., and Peattie, D. (1989) Phylogenetic meaning of the kingdom concept: an unusal ribosomal RNA from *Giardia lamblia*. *Science* **243**, 75–77.
63. Embley, T. M., and Hirt, R. P. (1998) Early branching eukaryotes? *Curr. Opin. Genet. Devel.* **8**, 655–661.
64. Philippe, H., Germot, A., Moreira, D. (2000) The new phylogeny of eukaryotes. *Curr. Opin. Genet. Dev.* **10**, 596–601.
65. Roger, A. J., and Silbermann, J. D. (2002) Mitochondria in hiding. *Nature* **418**, 827–828.
66. Tovar, J., Fischer, A., and Clark, C. G. (1999) The mitosome, a novel organelle related to mitochondria in the amitochondrial parasite *Entamoeba histolytica*. *Mol. Microbiol.* **32**, 1013–1021.
67. Williams, B. A., Hirt, R. P., Lucocq, J. M., and Embley, T. M. (2002) A mitochondrial remnant in the microsporidian *Trachipleistophora hominis*. *Nature* **418**, 865–869.
68. Biagini, G. A., Finlay, B. J., Lloyd, D. (1997) Evolution of the hydrogenosome. *FEMS Microbiol. Lett.* **155**, 133–140.
69. Hackstein, J. H. P., Akhmanova, A., Voncken, F., van Hoek, A., van Alen, T., Boxma, B., Yeo Moon-van der Staay1, S., van der Staay, G., Leunissen, J., Huynen, M., Rosenberg, J., and Veenhuis, M. (2001) Hydrogenosomes: convergent adaptations of mitochondria to anaerobic environments. *Zoology* **104**, 290–302.
70. Rotte, C., Stejskal, F., Zhu, G., Keithly, J. S., and Martin, W. (2001) Pyruvate : NADP Oxidoreductase from the mitochondrion of *Euglena gracilis* and from the apicomplexan *Cryptosporidium parvum*: A biochemical relic linking pyruvate metabolism in mitochondriate and amitochondriate protists. *Mol. Biol. Evol.* **18**, 710–20.
71. Horner, D. S., Heil, B., Happe, T., and Embley, T. M. (2002) Iron hydrogenases—ancient enzymes in modern eukaryotes. *Trends Biochem. Sci.* **27**, 148–153.
72. Yarlett, N., Orpin, C. G., Munn, E. A., Yarlett, N. C., and Greenwood, C. A. (1986) Hydrogenosomes in the rumen fungus *Neocallimastix patriciarum*. *Biochem. J.* **236**, 729–739.
73. Marvin-Sikkema, F. D., Rees, E., Kraak, M. N., Gottschal, J. C., and Prins, R. A. (1993) Influence of metronidazole, carbon monoxide, carbon dioxide and methanogens on the fermentative metabolism of the anaerobic fungus *Neocallimastix* sp. strain L2. *Appl. Environ. Microbiol.* **59**, 2678–2683.
74. Gray, M. W., Burger, G., and Lang, B. F. (1999) Mitochondrial evolution. *Science* **283**, 1476–1481.
75. Stechmann, A., and Cavalier-Smith, T. (2002) Rooting the eukaryote tree by using a derived gene fusion. *Science* **297**, 89–91.

76. Simpson, A. G. B., and Roger, A. J. (2002) Eukaryotic evolution: Getting to the root of the problem. *Curr. Biol.* **12**, R691–R693.
77. Baldauf, S. L., Roger, A. J., Wenk-Siefert, I., and Doolittle, W. F. (2000) A kingdom-level phylogeny of eukaryotes based on combined protein data. *Science* **290**, 972–977.
78. Bapteste, E., Brinkmann, H., Lee, J. A., Moore, D. V., Sensen, C. W., Gordon, P., Durufle, L., Gaasterland, T., Lopez, P., Müller, M., and Philippe, H. (2002) The analysis of 100 genes supports the grouping of three highly divergent amoebae: *Dictyostelium*, *Entamoeba*, and *Mastigamoeba*. *Proc. Natl. Acad. Sci. USA* **99**, 1414–9141.
79. Kobayashi, M., Matsuo, Y., Takimoto, A., Suzuki, S., Maruo, F., and Shoun, H. (1996) Denitrification, a novel type of respiratory metabolism in fungal mitochondrion. *J. Biol. Chem.* **271**, 16263–16267.
80. van der Giezen, M., Slotboom, D. J., Horner, D. S., Xue, G. P., Embley, T. M., and Kunji, E. R. S. (2002) Conserved properties of hydrogenosomal and mitochondrial ADP/ATP carriers: a common origin for both organelles. *EMBO J.* **21**, 572–579.
81. Voncken, F., Boxma, B., Tjaden, J., Akhmanova, A., Huynen, M., Verbeek, F., Tielens, A. G., Haferkamp, I., Neuhaus, H. E., Vogels, G., Veenhuis, M., and Hackstein, J. H. (2002) Multiple origins of hydrogenosomes: functional and phylogenetic evidence from the ADP/ATP carrier of the anaerobic chytrid *Neocallimastix* sp. *Mol. Microbiol.* **44**, 1441–1454.
82. Zhou, Z., Takaya, N., Nakamura, A., Yamaguchi, M., Takeo, K., and Shoun, H. (2002) Ammonia fermentation, a novel anoxic metabolism of nitrate by fungi. *J. Biol. Chem.* **277**, 1892–1896.
83. Yurimoto, H., Sakai, Y., and Kato, N. (2002) Methanol metabolism. In *Hansenula polymorpha: Biology and Applications*. (Gellissen, G. ed.). pp. 61–75, Wiley-VCH Verlag, Weinheim.
84. von Dohlen, C. D., Kohler, S., Alsop, S. T., and McManus, W. R. (2001) Mealybug β-proteobacterial endosymbionts contain γ-proteobacterial symbionts. *Nature* **412**, 433–436.
85. Roger, A. J. (1999) Reconstructing early events in eukaryotic evolution. *Am. Nat.* **154**, S146–S163.
86. Lang, B. F., Gray, M. W., and Burger, G. (1999) Mitochondrial genome evolution and the origin of eukaryotes. *Annu. Rev. Genet.* **33**, 351–397.
87. Stoebe, B., and Kowallik, K. V. (1999) Gene-cluster analysis in chloroplast genomics. *Trends Genet.* **15**, 344–347.
88. Moreira, D., Le Guyader, H., and Philippe, H. (2000) The origin of red algae and the evolution of chloroplasts. *Nature* **405**, 69–72.
89. McFadden, G. I. (2001) Primary and secondary endosymbiosis and the origin of plastids. *J. Phycol.* **37**, 951–959.
90. Cavalier-Smith, T. (2000) Membrane heredity and early chloroplast evolution. *Trends Plant. Sci.* **5**, 174–182.
91. Douglas, S., Zauner, S., Fraunholz, M., Beaton, M., Penny, S., Deng, L.-T., Wu, X., Reith, M., Cavalier-Smith, T., and Maier, U. G. (2001) The highly reduced genome of an enslaved algal nucleus. *Nature* **401**, 1091–1096.
92. Delwiche, C. W. (1999) Tracing the thread of plastid diversity through the tapestry of life. *Am. Nat.* **154**, S164–S177.
93. Archibald, J. M., and Keeling, P. J. (2002) Recycled plastids: a 'green movement' in eukaryotic evolution. *Trends Genet.* **18**, 577–584.
94. Stoebe, B., and Maier, U. G. (2002) One, two, three: nature's tool box for building plastids. *Protoplasma* **219**, 123–130.
95. Palmer, J. D. (2003) The symbiotic birth and spread of plastids: how many times and whodunit? *J. Phycol.* **39**, 4–12.
96. Martin, W., Rujan, T., Richly, E., Hansen, A., Cornelsen, S., Lins, T., Leister, D., Stoebe, B., Hasegawa, M., and Penny, D. (2002) Evolutionary analysis of *Arabidopsis*, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. *Proc. Natl. Acad. Sci. USA* **99**, 12246–12251.
97. Leister, D. (2003) Chloroplast research in the genomics age. *Trends Genet.* **19**, 47–56.
98. Hannaert, V., Saavedra, E., Duffieux, F., Szikora, J. P., Rigden, D. J., Michels, P. A., and Opperdoes, F. R. (2003) Plant-like traits associated with metabolism of *Trypanosoma* parasites. *Proc. Natl. Acad. Sci. USA* **100**, 1067–1071.
99. Huang, C.Y., Ayliffe, M.A., and Timmis, J.N. (2003) Direct measurement of the transfer rate of chloroplast DNA into the nucleus. *Nature* **422**, 72–76.
100. Fast, N. M., Kissinger, J. C., Roos, D. S., and Keeling, P. J. (2001) Nuclear-encoded, plastid-targeted genes suggest a single common origin for apicomplexan and dinoflagellate plastids. *Mol. Biol. Evol.* **18**, 418–426.
101. Yoon, H. S., Hackett, J. D., Pinto, G., and Bhattacharya, D. (2003) The single, ancient origin of chromist plastids. *Proc. Natl. Acad. Sci. USA* **99**, 15507–15512.
102. Funes, S., Davidson, E., Reyes-Prieto, A., Magallon, S., Herion, P., King, M. P., and Gonzalez-Halphen, D. (2002) A green algal apicoplast ancestor. *Science* **298**, 2155–2155.
103. Boekema, E. J., Hifney, A., Yakushevska, A. E., Piotrowski, M., Keegstra, W., Berry, S., Michel, K.-P., Pistorius, E. K., and Kruip, J. (2001) A giant chlorophyll-protein complex induced by iron deficiency in cyanobacteria. *Nature* **412**, 745–748.
104. Andersson, S. G. E., and Kurland, C. G. (1999) Origins of mitochondria and hydrogenosomes. *Curr. Op. Microbiol.* **2**, 535–541.
105. Doeller, J. E., Grieshaber, M. K., and Kraus, D.W. (2001) Chemolithoheterotrophy in a metazoan tissue: thiosulfate production matches ATP demand in ciliated mussel gills. *J. Exp. Biol.* **204**, 3755–3764.
106. Doeller, J. E., Gaschen, B. K., Parrino, V., and Kraus, D. W. (1999) Chemolithoheterotrophy in a metazoan tissue: Sulfide supports cellular work in ciliated mussel gills. *J. Exp. Biol.* **202**, 1953–1961.
107. Schütz, M., Shahak, Y., Padan, E., and Hauska, G. (1997) Sulfide-quinone reductase from *Rhodobacter capsulatus*. *J. Biol. Chem.* **272**, 9890–9894.
108. Griesbeck, C., Schütz, M., Schödl, T., Bathe, S., Nausch, L., Mederer, N., Vielreicher, M., and Hauska, G. (2002) Mechanism of sulfide-quinone reductase investigated using site-directed mutagenesis and sulfur analysis. *Biochemistry* **41**, 11552–11565.
109. Yong, R., and Searcy, D. G. (2001) Sulfide oxidation coupled to ATP synthesis in chicken liver mitochondria. *Comp. Biochem. Physiol. B.* **129**, 129–137.
110. Vande Weghe, J. G., and Ow, D. W. (1999) A fission yeast gene for mitochondrial sulfide oxidation. *J. Biol. Chem.* **274**, 13250–13257.
111. Theissen, U., Hoffmeister, M., Grieshaber, M., and Martin, W. (2003) Single eubacterial origin of eukaryotic sulfide:quinone oxidoreductase, a mitochondrial enzyme conserved from the early evolution of eukaryotes during anoxic and sulfidic times. *Mol. Biol. Evol.* **20**, (in press).
112. Martin, W. (1999) Mosaic bacterial chromosomes—a challenge en route to a tree of genomes. *BioEssays* **21**, 99–104.

113. Amend, J.P., and Shock, E.L. (2001) . Energetics of overall metabolic reactions of thermophilic and hyperthermophilic Archaea and Bacteria. *FEMS Microbiol Rev* **25**, 175–243.
114. Reeve, J. (2003) Archaeal chromatin and transcription. *Mol. Microbiol*. **48**, 587–598.
115. Iwabe, N., Kuma, K.-I., Hasegawa, M., Osawa, S., and Miyata, T. (1989) Evolutionary relationship of archaebacteria, eubacteria and eukaryotes inferred from phylogenetic trees of duplicated genes. *Proc. Natl. Acad. Sci. USA* **86**, 9355–9359.
116. Philippe, H., and Forterre, P. (1999) The rooting of the universal tree of life is not reliable. *J. Mol. Evol*. **49**, 509–523.
117. Andersson, J. O., and Roger, A. J. (2002) A cyanobacterial gene in nonphotosynthetic protists - An early chloroplast acquisition in eukaryotes? *Curr. Biol*. **12**, 115–119.
118. Baldauf, S.L., and Palmer, J.D. (1993) Animals and fungi are each other's closest relatives: congruent evidence from multiple proteins. *Proc. Natl. Acad. Sci. USA* **90**, 11558–11562.
119. Bapteste, E., and Philippe, H. (2002) The potential value of indels as phylogenetic markers: position of trichomonads as a case study. *Mol. Biol. Evol*. **19**, 972–977.
120. Cavalier-Smith, T. (1981) The origin and early evolution of the eukaryotic cell. In *Molecular and Cellular Aspects of Microbial Evolution* (*Symp. Soc. Gen. Microbiol*. Vol. 32) (Carlile MJ, Collins JF, Moseley BEB eds). pp. 33–84, Cambridge Univ. Press, Cambridge.
121. Horiike, T., Hamada, K., Kanaya, S., and Shinozawa, T. (2001) Origin of eukaryotic cell nuclei by symbiosis of Archaea in Bacteria is revealed by homology-hit analysis. *Nature Cell Biol*. **3**, 210–214.
122. Rotte, C., and Martin, W. (2001) Does endosymbiosis explain the origin of the nucleus? *Nature Cell Biol*. **8**, E173–174.