Primer

The co-evolution of life and Earth

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It has long been recognized that deciphering the relationship between the history of life on Earth and the history of the planet is a profound task. Recent technological innovations in both the earth and life sciences have made this task more tractable than ever before, leading to the emergence of the discipline of geobiology — the study of how organisms have influenced, and been influenced by, the Earth's environment. Along with enthusiasm for this new field, however, has come confusion, as geobiology combines highly specialized and historically separate fields. How does a sedimentologist communicate his/her problems to a cell biologist and vice versa? The fact that geobiology derives from two disparate scientific traditions — those of natural history and experimental science — can make identification of appropriate problems challenging. As C.P. Snow [1] recognized nearly a half century ago in his famous lecture 'The Two Cultures', communication between different disciplines often results in "a gulf of mutual incomprehension" that can be difficult to ford. Although Snow was referring to the divide that separates the humanities from the sciences, many of his insights can be applied to the divide that until recently has separated biology from geology.

In this Primer, we shall attempt to illustrate the compelling nature of geobiology by highlighting two geobiological problems. Our goal is to introduce molecular and cell biologists to this discipline, and make it clear just how much their skills can contribute to it and their questions benefit from it. We begin with a brief review of what is known about the geochemical evolution of the Earth. From there, we provide two examples of problems relevant to the co-evolution of life and Earth. The first example illustrates how a better understanding of biology — specifically, the distribution and function of sterol-like molecules in bacterial membranes — will inform our understanding of the rise of oxygen, arguably the most important event in the geochemical evolution of the Earth. The second example illustrates the counterpoint: how a better understanding of changes in the Earth's geochemistry over time can affect our interpretations of organelle evolution, specifically, the relationship between hydrogenosomes and mitochondria.

What we do and do not know about the Earth's geochemical evolution

Geologists use sedimentary rocks as probes to study the environments in which the primary sediment was originally deposited. A sandstone may contain structures useful for inferring water depth or flow velocities, while a rock formed primarily by precipitation may contain minerals or elemental distributions useful for inferring the abundances of certain chemical species in the precipitating fluid.

By studying relevant rocks deposited in different environments and at different times in Earth history, geologists have inferred a discontinuous, incomplete, but still useful record of the chemical evolution of the Earth's near surface environment. One of the major results of such investigation has been the realization that Earth history can be divided into three major intervals characterized by different environmental distributions of O_2: 4.6–2.4 billion years ago (Ga), 2.4–0.54 Ga and 0.54–0 Ga.

Pre-2.4 Ga

Most investigators agree that the atmosphere was essentially anoxic until 2.4–2.3 Ga [2] (Figure 1). Several lines of evidence support this conclusion, but one of the most important recent discoveries has been mass-independently fractionated sulfur in sulfide and sulfate minerals deposited pre-2.4 Ga. There are four stable isotopes of sulfur: ^{32}S, ^{33}S, ^{34}S and ^{36}S. Nearly all known processes that fractionate these isotopes do so in a mass-dependent manner. For instance, dissimilatory sulfate reduction tends to produce sulfide, which is depleted in the heavier isotopes of sulfur relative to the sulfate substrate; moreover, it tends to deplete ^{34}S about twice as much as ^{33}S (measured relative to ^{32}S). The only processes known to deviate significantly from this pattern are photochemical

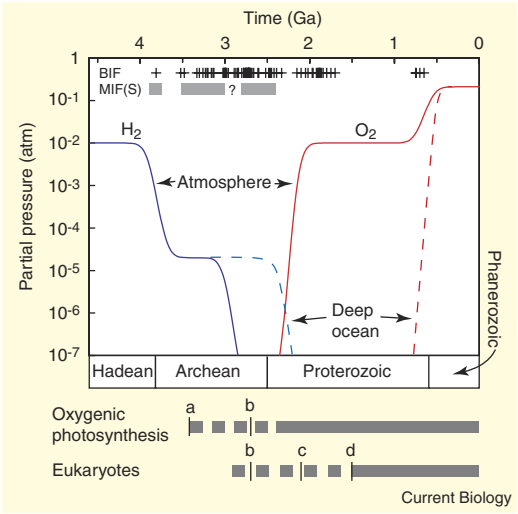

Figure 1. Geochemical evolution of the atmosphere and deep oceans.

Crosses represent occurrences of banded iron formation (BIF). Gray bars represent times for which mass-independent fractionation of sulfur (MIF(S)) has been observed in sulfide and/or sulfate minerals. Very few sedimentary rocks are known from 3.7–3.5 Ga. No MIF(S) has yet been observed in rocks 3.0–2.8 Ga. Solid lines indicate atmospheric abundances. Dashed lines indicate abundances in the deep ocean. H_2 abundances illustrated are speculative and unconstrained by geologic data. Four mileposts in the evolution of oxygenic photosynthesis and eukaryotes are noted: (a) the earliest evidence for anoxygenic (H_2-oxidising) photosynthesis; (b) the earliest known occurrence of steranes and 2-methylhopanes; (c) the first putative eukaryotic microfossils; and (d) the first known diverse acritarch assemblages (likely eukaryotic microfossils) in shallow marine sediments.

disproportionations — when a species of a given oxidation state reacts to give a mixture of species with both higher and lower oxidation states — of SO_2 and SO to S_8 and H_2SO_4.

The preservation of mass-independently fractionated sulfides and sulfates prior to 2.4 Ga implies that most sulfur deposited in sediments originated as volcanic SO_2, which was subsequently photolyzed. The resultant S_8 could not have been oxidized, otherwise the mass-independent signal in reduced and oxidized sulfur minerals would have been homogenized. Models of early atmospheric chemistry suggest that preservation of this signal required atmospheric O_2 abundances less than 10^{-5} times the present atmospheric level. Permanent loss of the mass-independent signal at 2.4–2.3 Ga probably coincides with the first time that atmospheric O_2 abundances rose permanently above that level.

There is less certainty, however, about when biological O_2 production began. In more recently deposited sediments, tracking the distributions of elements that form minerals with highly contrasting solubility in different redox states — such as Fe, Ce and, U — has been an important tool for determining the paleoenvironmental distribution of O_2. Unfortunately, this technique has produced ambiguous results in the search for ancient biological O_2 sources. For instance, U oxidation and reduction is highly sensitive to carbonate concentrations, and it is quite likely that carbonate was far more abundant in the early oceans than in today's oceans. Ce oxidation is potentially more revealing, but Ce enrichments and depletions have been difficult to correlate to biological activity.

Iron oxidation is recorded most prominently in 'banded iron formations' — iron-rich deposits frequently so large that they have been important economic sources of the metal (Figure 1). Iron in these units can be present in a range of minerals and average oxidation states, including siderite ($Fe^{II}CO_3$), magnetite ($Fe^{II}Fe^{III}{}_2O_4$) and hematite ($Fe^{III}{}_2O_3$). It is not clear what oxidized the banded iron formations. There are a number of possibilities, including: O_2 produced by oxygenic photosynthetic microbes; anaerobic photoautotrophic Fe-oxidizing microbes; ultraviolet light; and oxidants produced photochemically in the atmosphere. It is possible that more than one of these was important in various settings and times, but there is currently no empirical way of distinguishing mechanisms.

In contrast, there seems to be at least one case from the geologic record where it is possible to rule out O_2 production associated with photosynthesis. Microbial mats confined to shallow-water settings on a 3.42 Ga platform, apparently because they were constructed by photosynthetic organisms, did not oxidize Fe or Ce. From the distribution of redox-sensitive minerals and elements in associated rocks, Tice and Lowe [3] suggested that the most likely photosynthetic electron donor was H_2. At present, the most suggestive indication of early oxygenic photosynthesis is the identification of 2-methylhopanes and complex steranes in rocks deposited at 2.7 Ga. These biomarkers, however, are somewhat problematic (see Example 1), so dating the transition from anoxygenic photosynthesis to oxygenic photosynthesis remains an important challenge.

Instead of variations in O_2 abundance, it is possible that variations in H_2 abundance formed the plot of the most important biogeochemical story on the pre-2.4 Ga Earth. There is currently no geological way to estimate ancient H_2 concentrations, but models of early atmospheric chemistry suggest prebiotic atmospheric mixing ratios of 0.1–30% by volume [4]. The emergence and spread of methanogens, which convert H_2 and CO_2 to CH_4 and H_2O, would have significantly depleted atmospheric H_2 levels (Figure 1).

Further variation in atmospheric H_2 levels could have been induced by development of the first continents 3.2–3.0 Ga. The other two major episodes of continent formation, at 2.7–2.5 Ga and 1.0–0.7 Ga, were both followed by pulses of atmospheric oxidation, probably associated with burial of organic matter fixed by oxygenic photosynthetic organisms under sediment eroded from the new blocks of crust [5]. It is not clear that any net release of O_2 occurred following the 3.2–3.0 Ga episode; if it did, the effect was not permanent. Drawing an analogy with later episodes, however, suggests that net oxidation of the atmosphere might have occurred by removal of H_2. This removal would have been accomplished in a similar way to later additions of O_2 — by burial of organic matter produced by methanogens or anaerobic H_2-oxidizing photoautotrophs, preventing regeneration of consumed H_2. Thus, comparison to later episodes of Earth history leads to the intriguing possibility of a drop in atmospheric H_2 levels shortly after approximately 3.0 Ga. Deep marine H_2 abundances could have remained relatively high during this time.

2.4–0.54 Ga

The Earth's surface entered a new state 2.4–2.3 Ga. Mass-independent fractionation in sulfide and sulfate minerals disappeared permanently during this time, and the first widespread isotopic signals of sulfate reduction appeared. Isolated evidence for this process is present as early as 3.5 Ga, so this new stage probably reflects the first time that oceanic sulfate concentrations became non-limiting, most likely as a result of the new flux of oxidatively weathered sulfate to the oceans. New sulfate-rich conditions in the deep ocean probably drove H_2 concentrations there lower, as sulfate reduction became an important sink for organic matter and H_2.

It now seems possible that atmospheric O_2 levels did not rise sufficiently to oxygenate the deep ocean, but that sulfate levels were elevated enough to allow rampant sulfate reduction to

transform the deep ocean into a sulfidic environment (reviewed in [6]). Abundant sulfide effectively scrubbed the oceans of most ferrous iron, ending deposition of banded iron formations until widespread glaciations about 0.7 Ga briefly removed the weathering source of sulfate. Thus, for much of 2.4–0.54 Ga, the Earth's surface was probably divided into two contrasting chemical regimes: the atmosphere and surface ocean were weakly oxic, while the deep ocean was sulfidic. H_2 was most likely scarce in both locations except for microenvironments where biological sources overwhelmed diffusion and consumption.

0.54–0 Ga

Atmospheric O_2 levels began to rise about 0.54 Ga, probably due to effects associated with earlier continent formation, and were at present abundances shortly afterwards. Such levels are sufficient to overwhelm sulfate reduction in a well-mixed ocean, and, with the exceptions of times when oceanic circulation was inhibited, both the atmosphere and oceans have remained oxygenated since.

Example 1: How cellular and molecular biology can inform our understanding of geochemical evolution

The biological invention of oxygenic photosynthesis was a pivotal event in the evolution of both complex life and the chemistry of the Earth's surface. As described above, constraining the age of that evolutionary step remains a major goal for geologists and biologists alike. One promising approach to this question involves the study of hydrocarbon molecules known as 2-methylhopanes in the sedimentary record. Because of their unique carbon skeleton (Figure 2), these molecules can unambiguously be recognized as the molecular fossils of 2-methylhopanoids (2-MeBHPs) that are found in selected modern prokaryotes. Because cyanobacteria — the only prokaryotes that engage in oxygenic photosynthesis — are the only known quantitatively important source of 2-MeBHPs in the modern environment, earth scientists have inferred that 2-methylhopanes can be used as biomarkers for oxygenic photosynthesis itself [7]. Thus, Brocks *et al*. [8] interpreted the presence of 2-methylhopanes in sediments of the Archaean Fortescue Group as evidence that photosynthetically derived O_2 first appeared on Earth at least 2.7 Ga.

But does this make sense? As described above, a number of independent proxies indicate that a major global redox transition did not occur until roughly 400 million years later (2.3 Ga). If cyanobacteria were present and engaging in oxygenic photosynthesis at 2.7 Ga, why did it take approximately 400 million years to alter the surface redox state of the Earth? There may well be a good explanation for this lag, but if geochemists are incorrect in their assumption that 2-methylhopanes are biomarkers for oxygenic photosynthesis, then this paradox may be artificial.

A key question a molecular or cell biologist might ask is whether there is evidence that 2-MeBHPs and oxygenic photosynthesis are functionally related. Surprisingly, given the importance of this assumption, no such evidence exists. This is due to an inherent limitation in what organic geochemistry can teach, as recognized by experts in the field [9]. Organic geochemists are skilled in making precise measurements of complex organic compounds and in isolating them from messy environmental samples, but they are not able to determine the function of these compounds. At best, organic geochemistry can correlate the presence of particular molecules with particular organisms. Correlation is not causation, however, and plausibility is not proof. Here, the tools of molecular and cell biology can help. It is possible to determine whether particular membranes in a cell house 2-MeBHPs; it is possible to elucidate their biosynthetic pathway; and it is possible to study how cells behave when they can no longer make them. All of these questions (and more) have been explored in the context of sterols in eukaryotes, and there is good reason to believe the same experimental approaches

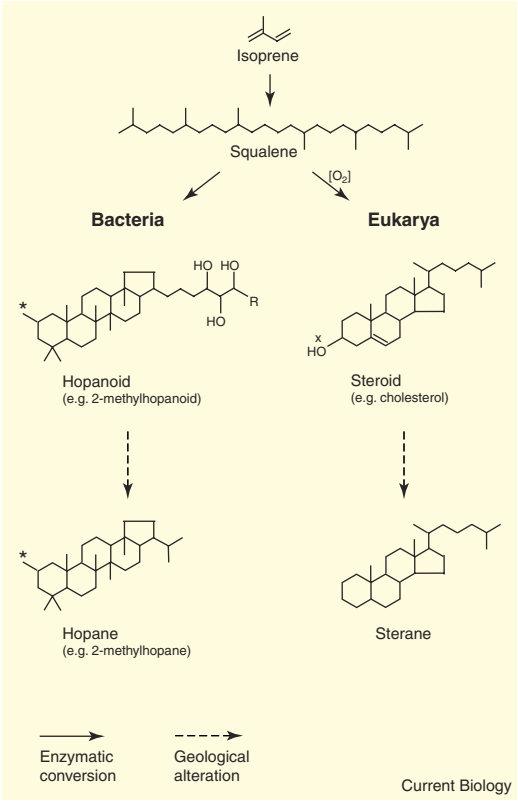

Figure 2. Biosynthesis and diagenesis of eukaryotic steroids and bacterial hopanoids.

Steroids and hopanoids are cyclic derivatives of the isoprenoid squalene. The best-studied steroid is the alcohol cholesterol. It has a tetracyclic ring structure that is hydroxylated at one of its rings (x) by an O_2-dependent mechanism. Cholesterol is an essential constituent of animal cell membranes, regulating membrane fluidity and organization. Bacterial hopanoids are pentacyclic compounds with an aliphatic tail that commonly contain between 4 and 6 hydroxyl groups and occasionally hexosesoramines. 2-methylbacteriohopanoids (BHPs) contain a characteristic 2-methylation site (*). During geological transformations in sediments (diagenesis) steroids and hopanoids get transformed into steranes and hopanes, losing unsaturated bonds and many of their functional groups.

will illuminate the biochemical function of 2-MeBHPs.

Although nothing is known about the function of 2-MeBHPs in particular, something is known about the functions of hopanoids more generally. Like eukaryotic sterols, hopanoids are thought to influence membrane fluidity and permeability [10]. Unlike sterols, however, hopanoid biosynthesis does not require molecular oxygen. Might 2-MeBHPs have been 'invented' in an anaerobic world, to serve a purpose related to membrane properties, and then later co-opted by cyanobacteria with similar cell biological needs? It is by now well established that structural modifications of sterols, including methylation of the polycyclic domain, have a dramatic impact on the biological function of higher organisms [11]. Recently, it has become apparent that sterols are capable of organizing heterogeneous microdomains within lipid bilayers. These microdomains, or lipid rafts, tend to sort proteins into clusters of functional significance. Specific structurally mediated lipid–lipid and lipid–protein interactions may be critical in determining the composition and subcellular localization of these rafts [12]. While the existence of lipid rafts has yet to be shown in bacteria, it seems possible that methylation of BHPs might be involved in the localization and activation of transmembrane proteins with a specific function.

Much remains to be done to characterize the occurrence of 2-MeBHPs in microbes with metabolically important differences — for example, in anaerobes versus aerobes or phototrophs versus heterotrophs — not to mention their biological function(s). Regardless of whether 2-MeBHPs are functionally related to oxygenic photosynthesis, understanding their role in modern organisms will greatly improve our interpretations of what their fossilized ancestors mean. Perhaps 2-MeBHPs are a marker for the evolution of a particular type of cell biological process, rather than a particular type of metabolism. Either way, the answer is interesting.

Example 2: How geochemistry can inform our understanding of organelle evolution

There is a long-standing debate over the evolution of early eukaryotes. It is generally accepted that the acquisition of the mitochondrion, an ATP-generating organelle, was a defining moment in this process. There is less agreement over the question of whether these eukaryotes were adapted to anaerobic or aerobic conditions.

The text book picture of a mitochondrion is that of an oxygen-respiring organelle, which is consistent with the widely held view that the earliest eukaryotes lived in an aerobic environment. This idea has been questioned by a number of findings. More than 30 years ago, a novel organelle, termed the hydrogenosome, was identified in the anaerobic flagellate *Trichomonas foetus* [13]. The hydrogenosome was named for its ability to gain energy from a fermentative metabolism that results in the release of molecular hydrogen. The organelle has since then been found in a limited number of eukaryotes that all share one feature: they live in anaerobic (or microaerobic) environments. Recently, genetic material was extracted from the hydrogenosome of *Trichomonas ovalis*; its DNA sequence suggests that hydrogenosomes and mitochondria are closely related [14].

Given these data, one might view hydrogenosomes as an obscure secondary adaptation to anaerobic environments, devoid of any significance for the evolution of early eukaryotes. In contrast to this view, Müller and Martin [15] proposed a provocative hypothesis in 1998 that emphasizes the crucial importance of the hydrogenosome's metabolism for the evolution of eukaryotes. According to the so-called 'hydrogenosome hypothesis', mitochondria and hydrogenosomes are derived from a common precursor that allowed for a facultative anaerobic lifestyle. This organelle is postulated to have contained an electron transport chain and ATPsynthase for aerobic respiration, still found in present day oxygen-consuming mitochondria, in addition to a set of enzymes that allowed for fermentative ATP production and H_2 release, still present in hydrogenosomes. A putative precursor of this organelle might have been a metabolically versatile Gram-negative bacterium, such as the α-proteobacterium *Rhodobacter sphaeroides*. Anaerobic H_2 production by this bacterial precursor is considered to have been the driving force for the symbiosis with its later host, a H_2-consuming methanogen (Figure 3A).

A strength of the hydrogenosome hypothesis is that it provides a metabolic rationale for the nature of the symbiosis. This allows for predictions about the type of environment that these symbionts must have encountered when they were in the process of forming the first eukaryote. What were the environmental requirements? First, the environment must have been anaerobic because methanogens (the hosts) are incapable of energy production in the presence of oxygen. Second, to ensure the dependency of methanogens to the hydrogen-producing proteobacteria, hydrogen levels must have been low.

Knowledge about the geochemical history of the Earth allows us to constrain the time frame of this symbiosis. When did the first eukaryotes arise? A variety of steranes have been extracted from 2.7 billion year old rocks and have been interpreted as a signature for eukaryotes [8]. But the production of sterols is not unique to eukaryotes, so this interpretation must be taken with care. The oldest microfossils that are generally accepted as demonstrating eukaryotic structures date back to 1.5 Ga [16]. In view of this, we need to focus our attention to at least 1.5 Ga, possibly as early as 2.7 Ga or even earlier. Do we find the environmental conditions as predicted by the hydrogenosome hypothesis before 1.5 Ga — a decrease of hydrogen levels in an anaerobic environment?

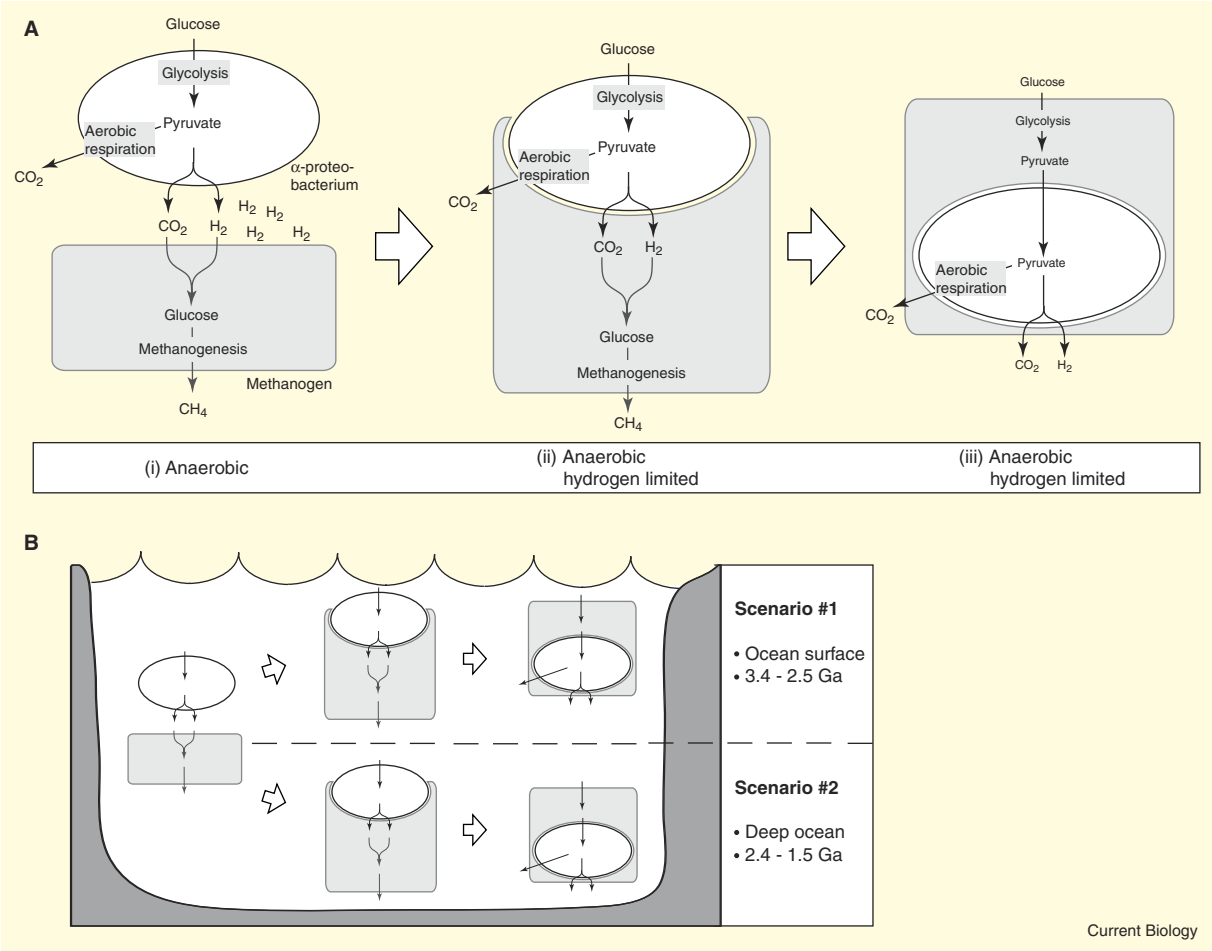

Figure 3. Model for the evolution of the first eukaryote according to the 'hydrogenosome hypothesis'.

(A) The 'hydrogenosome hypothesis': (I.) α-proteobacteria and methanogens entered a symbiotic relationship in an anaerobic environment. The proteobacteria were equipped with an electron transfer chain and an ATPsynthase for energy generation under aerobic conditions and a fermentative H_2-yielding metabolism for energy generation under anaerobic conditions. The methanogen yielded energy from methanogenesis. (II.) A dramatic drop of abiotic hydrogen concentrations constituted the selective pressure that drove methanogens into dependence with hydrogen-releasing proteobacteria. At one point a methanogen engulfed a proteobacterium and eventually incorporated it. (III.) The endosymbiosed proteobacterium 'handed over' the machinery for glucose uptake and glycolysis to its host. The resulting organelle was the precursor of mitochondria and hydrogenosomes being capable of aerobic respiration and hydrogen-generating fermentation. (B) Two scenarios that could have allowed eukaryotic evolution according to the hydrogenosome hypothesis (see text).

The geochemical evidence outlined in Figure 1 suggests that these conditions could have existed even twice: first, in shallow-water settings in equilibrium with the atmosphere at 3–2.5 Ga; and second, in deep-water settings 2.4–1.5 Ga. In either scenario anaerobic methanogens and facultative α-proteobacteria might have started their relationship in an anoxic environment with sufficient hydrogen for methanogens to live on. A dramatic decrease in hydrogen concentrations could then have constituted the key selective pressure on methanogens to enter an irreversible symbiosis with hydrogen producing α-proteobacteria, eventually taking them up as endosymbionts (Figure 3B).

It should be pointed out that the geochemical information summarized in Figure 1 is inferred from poorly spatially resolved global data. We cannot rule out — and, in fact, we even postulate — the existence of microenvironments that did not follow the global trends. But this does not mitigate the value that geological knowledge has for the evaluation of biological questions. In contrast, it is the synthesis of geological (biomarkers, microfossils and geochemical information) and biological (biochemical, genetic and phylogenetic) data that will bring us closer to a satisfying picture of the evolution of the first eukaryote.

Summary

We hope that these examples will convince that reader that the history of the evolution of life and the Earth can only be appreciated by deciphering their interdependencies. Molecular and cell biologists have tremendous potential to contribute to our understanding of the evolution of the Earth's near-surface environment by making concrete

links between biomarker structure and biochemical function. Similarly, geologists have much to offer evolutionary biology by helping constrain the time period and physical context of the appearance of new life forms.

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Revisiting Neandertal diversity with a 100,000 year old mtDNA sequence

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The cohabitation of Neandertals and modern humans in Europe about 35,000 years ago has stimulated considerable debate regarding hypothetical admixture. Recently, sequences of the hypervariable region-1 (HVR-1) of mitochondrial DNA (mtDNA) from 9 Neandertal specimens dated between 29,000 and 42,000 years ago from dispersed locations have revealed the genetic diversity of Neandertals around the time of the cohabitation [1–4]. The genetic signatures before and after contact with modern humans were found to be similar. They fall outside the range of modern human genetic diversity and show no specific affinity with modern or Paleolithic Europeans [5]. Such observations are generally taken as strong evidence for the 'Rapid replacement' model for the origin of modern humans [4,6], though further evidence is needed to completely exclude admixture [7].

The first presence of modern humans in Europe before 35,000 years ago as well as the survival of Neandertals beyond 30,000 years ago are still controversial issues [9]. Our goal was to recover a Neandertal sequence that unambiguously predates the cohabitation period. A comparison of this sequence with published Neandertal sequences might reveal either the long-time stability of the Neandertal mtDNA-pool or drastic modifications around the time of cohabitation. We, therefore, retrieved 123 bp of the mtDNA HVR-1 from a 100,000 year old Neandertal tooth from the Scladina cave (Meuse Basin, Belgium), which represents the most ancient Neandertal sample analyzed at the DNA level.

The experiments were conducted in a specific laboratory respecting the current authentication standards [10]. The extract was treated with uracil DNA-glycosylase (UDG) to excise deaminated cytosines formed after death, because they lead to artefactual GC→AT polymorphisms during PCR [11,12] and have already been shown to be present in sequences from Scladina fossils [13–15]. We took advantage of previously reported Neandertal sequences to design primers that favor the amplification of Neandertal DNA. PCR was never successful when fragments larger than 173 bp were targeted (Supplemental Data). We amplified four fragments spanning in total 221 bp of the HVR-1. Each PCR product was cloned and the final sequence was deduced from the consensus of 61 clones. Each position was found in at least two amplification products, except for the first 39 and last 59 nucleotides for which PCR replication was not possible. These nucleotides were consequently excluded from the sequence analyses. The remaining 123 bp (Figure 1) fulfilled all standards to guarantee the absence of DNA-damage-induced errors [10]. In addition, we are confident that the conditions in the Scladina cave favour DNA preservation, because an atomic C:N ratio typical of well-preserved collagen was found on the maxillary from the Scladina Neandertal [8], cave bear bones from the same excavation layer have already yielded authentic ancient DNA sequences [13,14] and 60,000–70,000 thousand year old nuclear DNA sequences were successfully amplified from woolly rhinoceroses from Scladina [15].

The Scladina Neandertal sequence has not been found among the 7161 human HVR-I sequences present in the HvrBase++ [16]. It appears more distantly related to the human than to the already reported Neandertal sequences (Figure 1). Of the 123 nucleotides considered, only one polymorphic site (at position 16258) has already been