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The Continuing Puzzle of the Great Oxidation Event

Review

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The rise of atmospheric O_2 was a milestone in the history of life. Although O_2 itself is not a climate-active gas, its appearance would have removed a methane greenhouse present on the early Earth and potentially led to dramatic cooling. Moreover, by fundamentally altering the biogeochemical cycles of C, N, S and Fe, its rise first in the atmosphere and later in the oceans would also have had important indirect effects on Earth's climate. Here, we summarize major lines of evidence from the geological literature that pertain to when and how O_2 first appeared in significant amounts in the atmosphere. On the early Earth, atmospheric O_2 would initially have been very low, probably $<10^{-5}$ of the present atmospheric level. Around 2.45 billion years ago, atmospheric O_2 rose suddenly in what is now termed the Great Oxidation Event. While the rise of oxygen has been the subject of considerable attention by Earth scientists, several important aspects of this problem remain unresolved. Our goal in this review is to provide a short summary of the current state of the field, and make the case that future progress towards solving the riddle of oxygen will benefit greatly from the involvement of molecular biologists.

Introduction

The emergence of molecular oxygen (O_2) as a significant constituent of the Earth's atmosphere was an epic event for both the biosphere and geosphere, and paved the way for the evolution of animal life. Indeed, in the search for signs of life on terrestrial-like planets beyond our solar system, planetary scientists tend to look first for O_2 [1]. It is thus ironic that when and how O_2 first accumulated on Earth remains a major puzzle. A number of recent reviews [2–7] have discussed the rise of atmospheric O_2, making the point that solving this puzzle requires an understanding not only of when the biological capacity to produce and utilize oxygen first appeared, but also how chemical and physical processes conspired to permit accumulation to its current level in the atmosphere.

Of all the biochemical inventions in the history of life, the machinery to oxidize water — photosystem II — using sunlight is surely one of the grandest. Not only did the ability to use water as a fuel provide early cyanobacteria with the advantage of an almost limitless supply of energy, but the production of O_2 as a waste product also profoundly changed the composition of the world's oceans, continents and atmosphere. The rise of atmospheric O_2 also had important indirect effects on Earth's climate. An anoxic atmosphere on the early Earth would likely have contained significant amounts of methane [8], which is a potent greenhouse gas. Increasing P_{O2} would have removed this methane greenhouse, possibly triggering dramatic cooling. Indeed, a series of near-global glaciations are believed to have occurred at about the same time that atmospheric O_2 first rose significantly [9]. The importance of these climate-active gases and their effects on evolution are discussed elsewhere in this issue [10].

Traditionally, when biologists have focused on the rise of O_2 their attention has been directed towards understanding the effects of O_2 on evolution and ecology. Thousands of articles and many books have been devoted to these topics [11]. For example, it is now well accepted that O_2 would at first have been toxic as well as a rich potential substrate for cellular respiration and biosynthesis. A dual-edged sword, it provided the driving force for the evolution of mechanisms both to detoxify and respire it [12,13]. Because oxygen detoxification mechanisms were presumably limited in an anaerobic world, a significant rise in O_2 may have caused a cataclysm in those organisms that were first exposed to reactive oxygen species. Organisms that could not adapt to O_2 were forced to remain in anaerobic niches, while others evolved detoxification mechanisms ranging from enzymatic (for example, catalase and superoxide dismutase) to behavorial (for example, clustering together) to survive in the newly aerobic environment.

Vestiges of this remain today: O_2 is a key player in signaling pathways found in many important developmental processes, including tumor cell growth [14] and nematode social feeding [15]. The advent of aerobic respiration was such a metabolic breakthrough — it is estimated to be about 16 times more efficient in generating ATP than anaerobic fermentation [1] — that some have argued this permitted the development of complex eukaryotic life [12,13], although others have proposed the first eukaryotic cell arose from an anaerobic symbiosis [16]. Whether the first phagocytotic progenitor of modern eukaryotes engulfed a smaller aerobically-respiring cell or something else, the eventual appearance of eukaryotic cells with the capacity to respire O_2 is generally agreed to have permitted the evolution of complex multicellular life and its eventual migration into terrestrial ecosystems [1,17–19].

In considering the history of O_2, molecular biology can play several roles. Perhaps the most obvious is through phylogenetic reconstructions of the evolutionary history of the machinery involved in oxygenic photosynthesis. Much has been written about this in recent years, and it is now generally accepted that the photosystems of cyanobacteria are an amalgam of different photosystems that originated in anoxygenic phototrophs that utilize reduced substrates, such as hydrogen, sulfide and ferrous iron as electron donors in their metabolism [20,21]. But while phylogeny can help us infer the relative timing of evolutionary events, it cannot tightly constrain the absolute timing of these events nor of environmental change. For this goal, we must turn to the rock record, and this is traditionally viewed as the exclusive domain of

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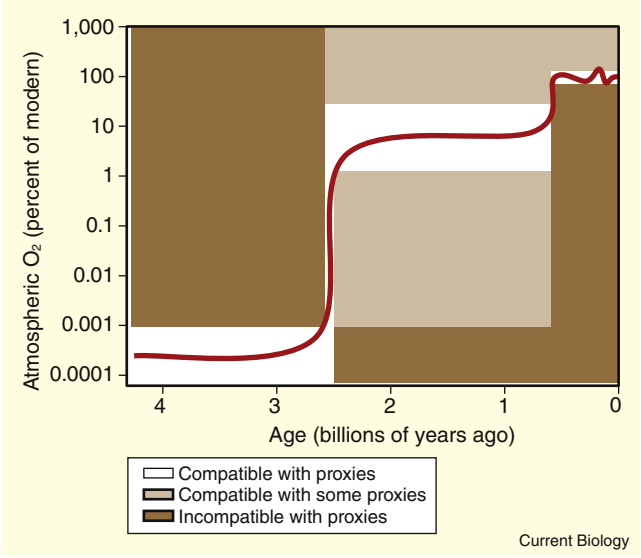

Figure 1. The prevailing view of atmospheric O_2 levels over geologic time.

Uncertainty in actual levels is far greater than that implied by the width of the red line. The three general plateaus in concentration correspond roughly (from left to right) to the Archaean, Proterozoic, and Phanerozoic eras of geologic time. The large increase at ~2.4 Ga is commonly known as the 'Great Oxidation Event'. Shaded regions indicate the rough constraints provided by geologic proxies discussed in the text. Note the logarithmic vertical axis. (Adapted with permission from [7].)

earth scientists. What is perhaps less well appreciated is that molecular biologists could significantly contribute to this goal as well, through efforts to identify and interpret the organic remains of ancient organisms that produced, respired or detoxified O_2. We shall briefly summarize the geological evidence for ancient changes in atmospheric O_2, highlighting where this record is ambiguous and where its interpretation could benefit from their involvement.

The Pattern and Timing of Oxygenation

While photosynthesis is the primary source of O_2, there are multiple environmental sinks, most significantly reduced carbon, iron and sulfur. The re-oxidation of biologic organic matter is the stoichiometric inverse of photosynthesis, so a first requirement for the net accumulation of O_2 at the Earth's surface is the burial of reduced organic carbon for geologic timescales. This burial process may have been much less efficient in the Precambrian than today due to a lack of biomineralization, which in the modern oceans provides ballast for sinking particles [22]. A second requirement is that the net production of O_2 must also have exceeded the delivery of reduced inorganic species from deep in the Earth by geologic processes. Fluxes of reduced Fe and S from the mantle, mainly at volcanoes and mid-ocean ridges, would also have been larger on the ancient Earth and presumably limited (or prevented) the accumulation of O_2 [23–25].

Although large uncertainties surround estimates of ancient O_2 levels, a general consensus now exists as to the rough pattern and timing of oxygenation (Figure 1). The evidence for this pattern is described in a number of excellent recent reviews [2,4,23,26], and is summarized below. The atmosphere of the earliest Earth was almost certainly anoxic, containing <0.1% of the present atmospheric level (PAL) and probably very much less (<10^{-5} PAL [27], but see [28] for an alternative viewpoint). Note that the evidence supporting this conclusion does not preclude the possibility of small oxygen oases in microbial mats or surface waters on the early Earth surface. Shortly after 2.45 billion years ago (denoted as '2.45 Ga'), atmospheric O_2 rose rapidly and substantially to probably a few percent or more of PAL. Due to its apparent speed and singular nature, this time interval has come to be known as the Great Oxidation Event.

The causes of the abrupt transition remain hotly debated. Published hypotheses include a change in the redox state of mantle gases [24,29], changes in marine nutrient supply [30] and/or reduced sinks for O_2 [31], a switch between two feedback-stabilized steady states [32], a decrease in atmospheric methane levels [33,34] and the evolution of oxygenic photosynthesis itself [35]. Regardless, by ~1.8 Ga atmospheric O_2 appears to have stabilized, possibly in the range of ~5–18% PAL [4], while the deep oceans remained anoxic. A second significant increase in atmospheric O_2 is postulated at around 0.6–0.8 Ga [36], and was accompanied by the oxygenation of the deep oceans and emergence of multicellular animals. By around 0.5 Ga, atmospheric O_2 was probably near its present level (21%), and has fluctuated around that value (15–35%) for the past half billion years (reviewed by [37]). This review focuses primarily on the evidence for, and consequences of, the first Great Oxidation Event.

Geologic Proxies for Atmospheric O_2

There are no direct records of atmospheric composition, such as air bubbles trapped in ice, that extend back more than ~1 million years. Instead, the history of atmospheric O_2 must be inferred from a variety of geologic and geochemical proxies. Most of these record redox-sensitive processes that provide only threshold values for O_2, for example indicating that oxygen was either above or below a specific level. None of them are directly proportional to P_{O2}. A second set of proxies record the presence of biota with specific metabolic capabilities, relating to the ability to produce or utilize O_2. These also yield threshold values for atmospheric O_2, with the added complication that they could reflect biota living in small, specialized habitats that were not representative of the greater environment. Thus, to obtain an accurate record of the appearance and accumulation of atmospheric O_2 over time, Earth scientists must piece together information from diverse proxies spanning both time and space. The major lines of evidence assembled thus far are summarized below and in Figure 2.

Fluvial Deposits of Redox-Sensitive Minerals

Sediments deposited by streams can be recognized from their characteristic grain shape and size distribution, and are necessarily deposited in contact with the atmosphere. When they contain detrital grains of redox-sensitive minerals that are readily oxidized by O_2, a low P_{O2} can be inferred. Pyrite (FeS_2), uraninite (UO_2) and siderite ($FeCO_3$) are the most commonly employed minerals, and yield threshold values of <0.1, <0.01 and <0.001 PAL O_2, respectively [2]. The primary difficulty is that such deposits are quite rare in old rocks, and must be examined in unweathered exposures (generally drill core or mine workings). They have been previously reported from the Witswatersrand (~3.1 Ga) in South Africa [38,39] and from the Pilbara Craton (2.7–3.2 Ga) of Australia [40].

Mobilization of Redox-Sensitive Trace Metals

Many trace metals are highly soluble in water — and thus mobile in the environment — in just one redox state. Elements such as Fe and Mn are soluble only in reduced form, while U, Mo, Re and Ce are soluble only when oxidized. The depletion of certain elements from ancient soils ('paleosols') formed by subaerial weathering, or their accumulation in marine sediments, can thus be used to infer P_{O2} in the atmosphere. The paleosol record indicates that those older than ~2.4 Ga exhibit significant loss of Fe (implying low O_2 with a threshold around $<10^{-3}$ PAL), while those younger than ~1.8 Ga show little loss of Fe (>0.1 PAL; reviewed by [41]).

Marine sediments also contain a record of redox-sensitive elements, albeit one that records atmospheric composition indirectly. Kopp *et al.* [35] interpret the massive accumulations of Mn in the ~2.2 Ga marine Hotazel formation as evidence for a substantial rise of O_2 which would have titrated Mn out of the oceans. More recently, Anbar *et al.* [42] have demonstrated a transient increase in the abundance of redox-sensitive Mo and Re in the 2.5 Ga Mount McRae Shale, which they interpret as an increase in oxidative weathering at least 50 million years prior to the Great Oxidation event. Pushing further back into geologic time, Rosing and Frei [43] have argued that an apparent excess of U relative to Th (whose solubility is not redox sensitive) in Isua metasediments (~3.8 Ga) indicates the presence of photic-zone O_2. This conclusion remains controversial, mainly because later Archaean sediments do not preserve the same signal [2].

S Isotope Fractionations

Numerous biogeochemical processes alter the relative abundances of the stable isotopes of sulfur (^{32}S, ^{33}S, ^{34}S and ^{36}S). These isotopic 'fractionations' can then be recorded in geologic materials, generally sulfide and sulfate minerals. Two aspects are relevant to the history of O_2. First, the relative depletion of ^{34}S in sulfides is controlled mainly via the process of bacterial sulfate reduction (reviewed by [44]). When sulfate is abundant, bacterial sulfate reduction yields a substantial depletion of ^{34}S in H_2S (and thus precipitated pyrite) relative to SO_4^{2-}. When sulfate is limited, however, the fractionation decreases to nearly zero because the sulfate is completely consumed. Thus, a global increase in the fractionation of ^{34}S between sedimentary pyrite and sulfate at around 2.3–2.4 Ga (Figure 2) is commonly interpreted as evidence for an increasing marine sulfate reservoir and, by extension, oxidative weathering of sulfide minerals on continents to supply that sulfate [45]. The P_{O2} threshold for this effect is estimated at <0.4% PAL [4].

The second relevant aspect is that virtually all biogeochemical processes fractionate ^{33}S, ^{34}S and ^{36}S from ^{32}S by amounts that are proportional to their relative masses. However, the photolysis of SO and SO_2 by near-UV radiation can produce fractionations that do not follow this proportional rule, and can be recorded in sedimentary sulfates and sulfides [46]. Because the process requires a lack of both atmospheric ozone and O_2, the appearance of these 'mass-independent' fractionations in the geologic record is considered strong evidence for an anoxic atmosphere with a threshold level around $<10^{-5}$ PAL [27]. Evidence for mass-independent fractionation is widespread in rocks older than ~2.45 Ga, and disappears entirely after ~2.3 Ga [47–50], providing what is probably the tightest constraint on the timing of the Great Oxidation Event. Farquhar *et al.* [51]

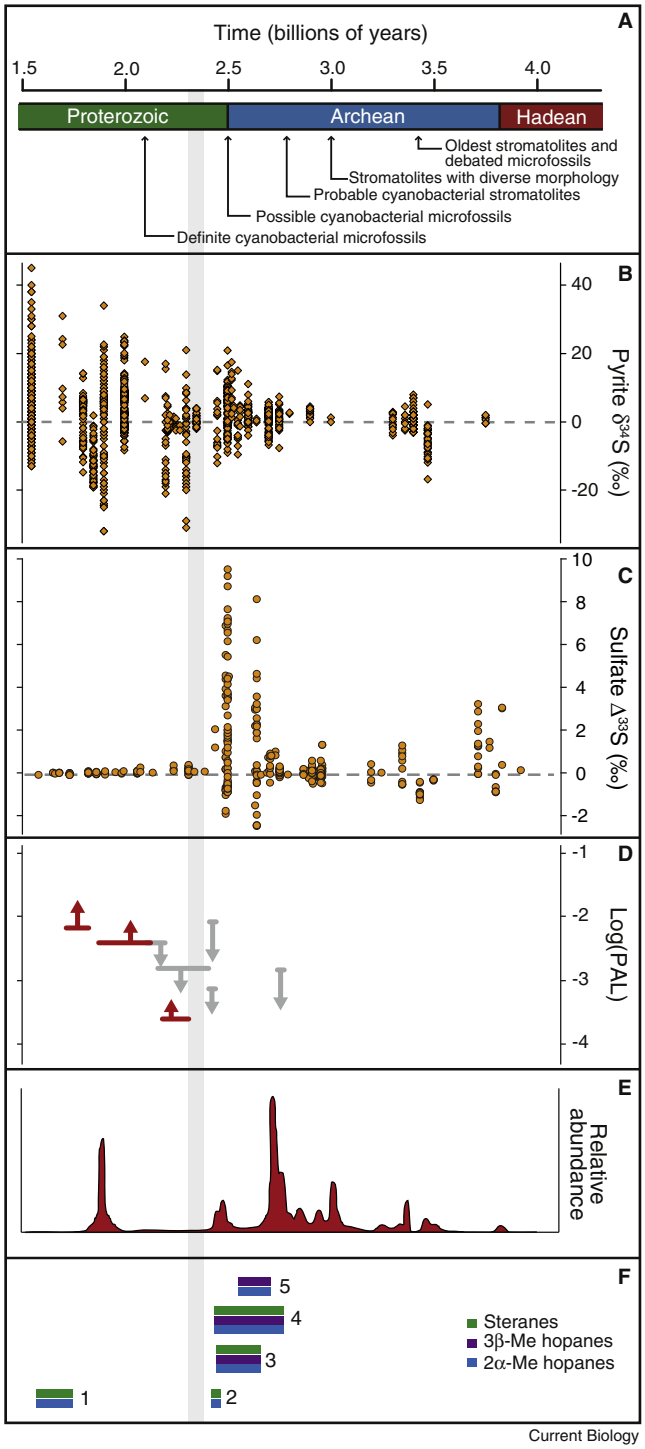

Figure 2. Geologic proxy records for oxygenic photosynthesis and the rise of atmospheric O_2.

(A) Geologic timescale and paleontological constraints (after [4] and [100]). (B) Fractionation of ^{34}S in pyrite [101]. (C) Mass-independent fractionation of S isotopes [51]. (D) Constraints from Fe content of paleosols (after [41]). Red arrows indicate Fe retention (oxidized), gray arrows iron loss (reducing). (E) Accumulation of banded iron formations [102]. (F) Reported detections of organic biomarkers. Numbers indicate sources: 1, Macarthur Group [103]; 2, Matinenda Fm [70]; 3, Transvaal Supergroup [71]; 4, Hamersley Group and Fortescue Group [73]; 5, Hamersley Group; [74]. The syngeneity of the biomarkers reported by Brocks *et al.* [73] has recently been disputed by [96].

have argued that changes in the specific mass-independent fractionation relationships in rocks from the Mesoarchaean (~2.8–3.2 Ga) indicate low levels of atmospheric O_2 well in advance of the Great Oxidation Event. A recent report of anomalous sulfur isotopic fractionation during thermochemical sulfate reduction by amino acids [52] may complicate the picture somewhat. However, the magnitude of mass-independent fractionation signals indicated by this report are very small relative to those preserved in the rock record.

Banded Iron Formations

Thick geologic deposits containing a high abundance of Fe(III) oxide, known as banded iron formation, are widespread in the Archaean, and largely absent after ~2.4 Ga (with significant exceptions at ~1.8 Ga and again at ~0.73 Ga). They are presumed to form in anoxic oceans with high dissolved Fe(II) via the oxidation and precipitation of iron (for example, [53,54]). Their presence is confidently interpreted as evidence for anoxic oceans in the Archaean, although the mechanism of oxidation remains disputed. Mixing with oxygenated surface waters and/or direct precipitation by anoxygenic bacterial phototrophs are both possible [55].

Fossils

Rocks older than ~1.8 Ga do not contain any macroscopic fossils that can be used to confidently infer the presence of significant O_2. However, two types of microfossils have been used to argue for an early origin of oxygenic photosynthesis. First, dome-like sedimentary structures known as 'stromatolites' are commonly preserved in Archaean rocks as old as 3.45 Ga [56]. They are often, though not always, interpreted as resulting from sediment trapping and binding in microbial mats (for example, [57]), and many contain preserved microbial filaments. While most are equivocal with respect to their biotic origins, large (up to 3 m) stromatolites in the lacustrine Tumbiana Fm (~2.7 Ga) contain exceptionally well-preserved microbial filaments that exhibit evidence of phototaxis [58]. Buick [2,58] has argued that the bacterial filaments were likely oxygenic photoautotrophs, but the possibility of anoxygenic photosynthesis based on H_2 cannot be excluded, especially in light of evidence for H_2-based photoautotrophy in putative microbial mats from the ~3.4 Ga Buck Reef Chert in South Africa [59].

Isolated, individual microfossils have been reported from two localities within the 3.49 Ga Dresser Fm [60,61]. Based on morphologic similarities to modern species, they have been interpreted as cyanobacteria that presumably carried out oxygenic photosynthesis [62]. However, given that they are poorly preserved and consist of little more than carbonaceous spheres and rods, this interpretation has been met with healthy skepticism (for example, [63]).

Organic Proxies

Certain structurally complex organic molecules, mainly lipids, can be preserved in sediments over geologic timescales. While some chemical alterations occur, they can often be recognized from their characteristic carbon skeletons and are thus termed 'molecular fossils' or, equivalently, 'biomarkers' [64]. When such biomarkers can be confidently related to parent organisms that either produce or require O_2, they can be used to document the history of O_2 [65]. Currently, the most widely employed organic proxies for this purpose are 2-methylhopanes, 3-methylhopanes, and steranes. Their presumed biotic sources are, respectively, cyanobacteria (producers of O_2 [66]), aerobic methanotrophs (obligate aerobes [67]) and eukaryotes (which require O_2 to biosynthesise sterols [68]).

These biomarkers have now been detected in fluid inclusions from the Canadian Matinenda Fm (~2.4 Ga [69,70]), and in solvent extracts from the South African Transvaal Supergroup (2.46–2.67 Ga [71]) and Australian Fortescue and Hamersley Groups (2.56–2.72 Ga [66,72–74]). Biomarkers lie at the intersection between biology and geology, and in our opinion represent one of the more promising yet least developed lines of evidence regarding the history of oxygen. And it is here that molecular biologists stand to make major contributions. We focus in the remainder of this review on the characteristics and interpretation of biomarkers.

Biomarkers: An Overview

The fact that life is so profoundly affected by oxygen makes it one of the most sensitive and selective detectors of O_2. As a concrete example, a brief survey of microbial diversity in almost any modern environment would instantly reveal the extent of oxygenation in that environment. There are thus many reasons to expect that the molecular fossils of ancient life, deposited as sedimentary organic matter, should contain abundant information about the history of O_2. However, the utility of biomarkers depends critically on our ability to relate a particular molecular structure to the identities and/or metabolic capabilities of the organisms that produced them. They are most useful when they are both highly specific and chemically stable so that they can persist in nature, either unchanged or modified in predictable ways, for long periods of time [75]. Unfortunately most biopolymers, including DNA and proteins, are highly specific but poorly preserved, generally surviving for only days to years after cell death [76]. In contrast, many lipids are well preserved but lack specificity, consisting of little more than linear aliphatic chains. Most work has therefore focused on a subset of isoprenoid lipids that are both structurally complex and highly recalcitrant to degradation. Chief among these are triterpenoids such as steroids and hopanoids, and certain carotenoid pigments such as isorenieratene and okenone [77–79].

Biomarkers carry multiple and interwoven threads of information by way of their primary chemical structures, the structural and stereochemical transformations they undergo upon release into the environment (diagenesis), and the isotopic compositions of their constituent atoms [80–82]. Of all the classes of biomarkers studied to date, lipids derived from photosynthetic organisms are the most abundant in ancient sedimentary organic matter. These comprise photosynthetic pigments (carotenoids and chlorophyll-derived tetrapyroles and their esterifying alcohols), hopanoid triterpenoids derived from bacteria, including cyanobacteria, and the sterols, long-chain ketones and hydrocarbons from eukaryotes, including algae (Figure 3). Of these, the hopanoids and sterols, which likely have a shared evolutionary origin [68,83], have been proposed as biomarkers that can inform us, with varying degrees of specificity and confidence, about the production and consumption of O_2 [65].

Sterols are essential components of the membranes of eukaryotes and the most comprehensively studied and best understood class of biomarkers with respect to biosynthesis and physiological function [84,85], biological distributions [86] and geochemical record [77,87]. Oxygen availability is not only generally accepted as an absolute

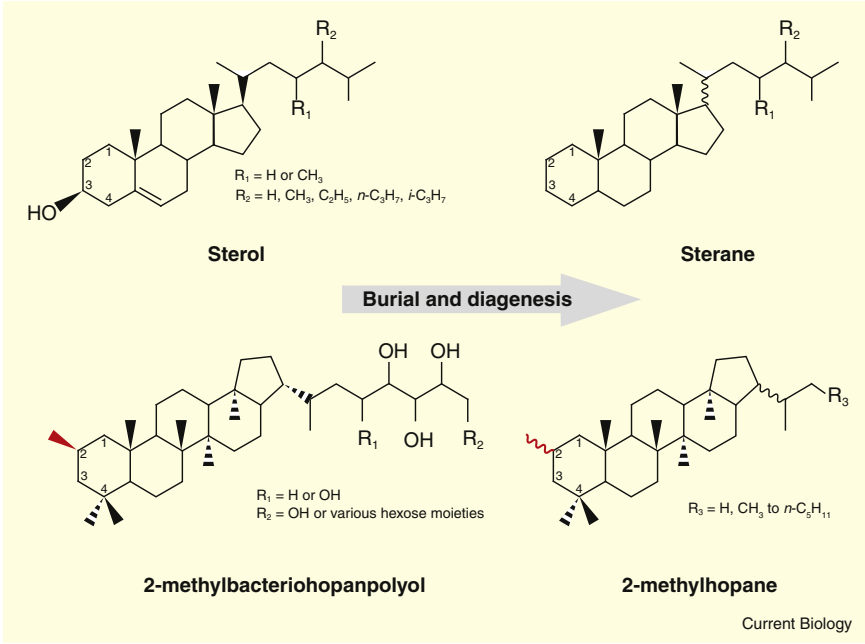

Figure 3. Generalized structures of the two classes of cyclic triterpenoid lipids discussed in the text: steroids (top) and hopanoids (bottom).

Left-hand structures represent the biologic precursor molecules, right-hand structures are the 'molecular fossils' identified in sediments. Sterols contain numerous combinations of unsaturation and alkylation at multiple positions, only a few of which are shown. Alkylation is generally preserved in the sedimentary record, while unsaturation is not. The biosynthesis of sterols requires O_2 in all known organisms. Hopanoids exhibit less structural diversity in the carbon skeleton, but more complicated patterns of functionalization in their alkyl 'tail'. This functionalization is not preserved in sediments, leading to a homologous series of compounds with varying *n*-alkyl hydrocarbon tails. Hopanes methylated on ring A at C-2 (shown) and C-3 are commonly interpreted as biomarkers for cyanobacteria and aerobic methylotrophs, respectively.

requirement [85] for sterol biosynthesis (although the possibility of an oxygen-independent pathway has been proposed [88]), the process is oxygen-intensive with 11 molecules of O_2 required to produce one molecule of cholesterol [65].

In contrast, O_2 is not required for any step in the biosynthesis of hopanoids, although paradoxically most known hopanoid-producing bacteria are aerobes [89]. Recently, however, culturing work [90–92] and bioinformatic predictions of hopanoid biosynthetic capability [93] have revealed that anaerobes can produce them too. Hopanoids bearing a methyl substituent on the A-ring, either at C-2 or C-3, are of particular interest with respect to environmental O_2 because of their apparently restricted biotic origins. 2-Methylhopanoids have been interpreted as indicators of cyanobacteria (and indirectly, oxygenic photosynthesis) because surveys found significant production of these molecules to be limited to the cyanobacterial clade [66]. The recent discovery of equally great 2-methylhopanoid production by an anoxygenic phototroph brought this into question [91], however, and we elaborate on this below.

Nevertheless, 3-methylhopanoids are still thought to reflect oxygen-requiring processes as the organisms known to produce their progenitors (acetic acid bacteria, *Acetobacter* sp.) and microaerophilic methanotrophic bacteria (Type 1 methanotrophs [67]) require oxygen for their metabolism. Isotopic analyses can be used to distinguish 3-methylhopanoids originating from these two groups in natural samples and in chemical fossils because, when their isotopic compositions have been measured, 3-methylhopanoids invariably have been found to have very low δ13C values (< –50 ‰), consistent with an origin from microaerophilic, methane-consuming Gammaproteobacteria (and inconsistent with an origin from *Acetobacter*) [94,95]. Of course, it may be that organisms will be found to make them under strictly anaerobic conditions in the future, which would be a confounding factor.

Over the approximately sixty years of biomarker studies, three problems have persistently bedeviled their interpretation. First, uncertainty regarding whether the biomarkers were deposited synchronously with the sediments in which they are found (for example [96]). Second, where a progenitor–product relationship is suspected, the possibility that the diversity of potential biotic sources has not been adequately sampled (this problem is particularly acute given that very little of modern microbial diversity has been cultured in the laboratory [97]). And third, the possibility that production of progenitor molecules is only expressed under some particular set of growth conditions, thus making it difficult to identify organisms that can produce them today. Beyond the questions of whether biomarkers are syngenetic and have been assigned an appropriate phylogenetic distribution lies the more profound question of their physiological function. While we can never be sure that what we learn from modern organisms is applicable to their ancient relatives, understanding the physiological function of biomarker progenitors would significantly enhance our ability to interpret biomarkers in the rock record. We illustrate these challenges and opportunities with a case study of 2-methylhopanoids.

Opportunities for Molecular Biologists: Hopanoids as a Case Study

Along with other geologic proxies, the presence and patterns of occurrence of 2-methylhopanes in ancient sediments has been used to promote the argument that oxygenic photosynthesis evolved well before the Great Oxidation Event. The parent molecules of 2-methylhopanes are 2-methylbacteriohopanepolyols (2-MeBHPs), a class of methylated hopanoid molecules (Figure 3) that were thought to originate mainly from cyanobacteria [66]. Because cyanobacteria are the only bacteria capable of oxygenic photosynthesis, 2-MeBHPs were also thought to be good biomarkers for oxygenic photosynthesis itself [66]. However, the discovery of significant production of these molecules in the anoxygenic phototroph *Rhodopseudomonas palustris* challenged this interpretation [91]. Not only did this study raise the possibility that 2-MeBHP production was more widely phylogenetically distributed than had been thought, it

demonstrated that the production of 2-MeBHPs was contingent upon growth conditions.

Of course, it is also possible that *R. palustris* is the exception and not the rule, and that cyanobacteria are indeed the primary sources of these compounds in the marine realm [2]. To more confidently make phylogenetic claims about the capacity of any particular group to make (or not make) 2-MeBHPs, it would help to have a means to predict this ability that is independent of culturing. And it is here that molecular biologists can help address the aforementioned problems that have confounded biomarker researchers. Through genetic analysis of model organisms, it is possible to identify the genes that catalyze specific steps in 2-MeBHP biosynthesis. Once identified, these sequences can be used to identify potential 2-MeBHP producers from DNA from sequence databases and/or environmental samples. Armed with such a tool, culture work would be more informed, both with respect to which organisms might be worth testing (for example, do organisms X and Y have the biosynthetic genes of interest?) as well as for the culture conditions that elicit their production (for example, are the biosynthesis genes expressed?).

Beyond developing a sequence-based tool to identify potential 2-MeBHP producers, an opportunity exists for molecular biologists to elucidate the biological function of 2-MeBHPs. Cell biological studies to localize the distribution of 2-MeBHPs within cells would be informative, as would *in vitro* studies to gain insight into how these molecules integrate into membranes, and whether they associate preferentially with other cellular components (specific proteins, for example). By constructing deletion mutants that no longer produce 2-MeBHPs, *in vivo* studies of these mutants could be performed to determine the physiological role(s) of these molecules. Collectively, experiments of this type would help to establish whether there is any functional link between photosynthesis and 2-MeBHPs. It may be that 2-MeBHP will be shown to have no direct connection to photosynthesis, in which case their use as a biomarker for oxygenic photosynthesis would be greatly weakened. Alternatively, we might learn through studies of 2-MeBHPs regulation which, if any, environmental factors induce or inhibit their expression in different organisms. If consistent patterns emerged from this type of analysis, it is conceivable that, rather than serve as biomarkers of oxygenic photosynthesis, 2-MeBHPs might more appropriately be used to interpret the Earth's paleoenvironment.

Conclusions

Given that organic molecular fossils comprise one of the potentially most powerful records of ancient biological processes, it is almost a truism to state that understanding the function and diversity of their modern progenitors is essential to interpreting evolutionary events. Nevertheless, until recently, few molecular biologists have been involved in their interpretation or identification. Our hope is that this review will stimulate interest by molecular biologists and biochemists in problems related to the evolution of metabolism, such as the capability to split water in photosynthesis and produce O_2. Until now, Earth scientists have focused almost exclusively on hopanes and steranes as organic proxies for the rise of O_2, but as more is understood about the molecular basis of photosynthesis, it may be that novel biomarkers will emerge that are equally, or more, informative. There are numerous 'orphan biomarkers' — novel fossil hydrocarbons that have no known biological progenitor — which, as we become more informed about lipids in modern cells, might one day gain meaning.

Conversely, by understanding which molecules have good preservation potential, biochemists will be able to propose potential biomarkers that organic geochemists can then search for. Just as Earth scientists stand to benefit from recruiting biologists to help solve their problems, biologists have equally great opportunities to benefit from Earth scientists. The roles lipids play in recruiting particular proteins to membrane micro-domains and contributing to membrane curvature are exciting frontiers in eukaryotic cell biology [98], but are relatively unexplored in bacteria and archaea [99]. In choosing a class of cellular constituents to study, all else being equal, why not pick those whose relevance spans billions of years?

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