

## THE EVOLUTION OF THE SULFUR CYCLE

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**ABSTRACT.** There are 2 principal avenues of inquiry relevant to reconstructing the history of the sulfur cycle. One avenue relies on the comparison of molecular sequences derived from biologically essential proteins and genetic material. Most sequence information is available from the small subunit of the rRNA molecule and from these sequences a Tree of Life emerges providing a reconstruction of the evolutionary relationships among organisms. Near the root of the tree are numerous bacteria metabolizing sulfur species including organisms living from dissimilatory elemental sulfur reduction, dissimilatory sulfate reduction, and anoxygenic photosynthesis. These metabolisms are likely very ancient. Many of the deep-branching bacteria of the sulfur cycle are active at very high temperatures (hyperthermophiles) and are commonly found in modern sulfide-rich hydrothermal systems. One can imagine a primitive early Earth terrestrial ecosystem housed around active hydrothermal areas with anoxygenic photosynthesis producing organic matter and oxidized sulfur species. These oxidized sulfur species could have been used as electron acceptors in the mineralization of organic matter, completing the carbon cycle. The evolution of oxygenic photosynthesis provided for dramatically increased rates of carbon production, and a much wider range of ecosystems for both carbon production, and carbon oxidation. Either associated with, or following, the evolution of oxygenic photosynthesis is the emergence of lineages housing most of the bacteria of which we are familiar, including most of the bacteria of the sulfur cycle.

The geologic record can provide direct evidence for the state of chemical oxidation of the Earth-surface, with possible indications of when specific bacterial metabolisms first occurred. We offer the following scenario for the evolution of the Earth-surface environment based on the available geological evidence. By 3.5 Ga anoxygenic photosynthesis was established and provided a weak source of sulfate to the global ocean with sulfate concentrations likely  $\ll 1$  mM. In some instances locally high concentrations of sulfate could accumulate and precipitated as evaporitic sulfate minerals. There is no compelling evidence for sulfate reduction at this time. The first evidence for sulfate reduction is found between 2.7 and 2.5 Ga, and the first evidence for oxygen production by oxygenic photosynthesis is found at around 2.8 Ga. Even so, levels of seawater sulfate remained low, below 1 mM, and did not increase to  $> 1$  mM until around 2.3 Ga. This increase in sulfate levels may have been promoted by a rise in atmospheric oxygen concentration at this time. Throughout the Archean and early Proterozoic the deep oceans contained appreciable concentrations of dissolved ferrous iron, and banded iron formations (BIFs) were a common form of chemical sediment.

Sulfate levels increased slowly, and by 1.8 Ga sulfate concentrations were sufficient to increase rates of sulfate reduction to greater than the delivery flux of iron to the oceans. Sulfide accumulated and precipitated ferrous iron from solution. It is proposed that the oceans remained sulfide-rich until the Neoproterozoic, where renewed deposition of banded iron formations occurred at around 0.75 Ga. It is possible that during the Neoproterozoic, decreased carbon production resulted from an ice covered "Snowball Earth" reducing rates of sulfate reduction below rates of iron delivery to the oceans, promoting BIF formation. At around this time high carbon burial rates increased levels of atmospheric oxygen to  $> 10$  percent present-day levels, promoting the widespread oxidation of marine surface sediments and an evolutionary radiation of sulfide oxidizing bacteria.

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<sup>1</sup> Throughout this text the term bacteria refers to prokaryotic organisms from both of the Domains Bacteria and Archaea. When written as Bacteria, organisms of the Domain Bacteria are referred to.

## DEDICATION

Bob Berner has invested much of his career in establishing the basic principles by which we understand both the modern and ancient cycling of sulfur. His contributions have been many, but highlights include: the first detailed study of sulfur diagenesis in marine sediments (Berner, 1964a), the first model from which the dynamics of sulfur respiration could be quantified (Berner, 1964b), the first detailed studies on the mechanisms of pyrite formation in modern marine sediments (Berner, 1970), and early insights into the factors controlling pyrite burial rates in both modern (Berner, 1982) and ancient (Berner and Raiswell, 1983; Berner, 1984) sediments. Bob has been a strong advocate of multidisciplinary approaches to solving geological problems as evidenced, for example, by his recent models of carbon dioxide regulation. These models include a wide range of considerations including the history of solar luminosity, rock-weathering kinetics, global tectonics, sea-floor spreading rates, and the evolution and subsequent proliferation of vascular land plants (Berner, 1994). It is in this spirit of Bob's multidisciplinary approach to science that this contribution is offered.

## GLOSSARY

- aerobe: an organism that metabolizes oxygen.
- amphibolite grade: high-grade metamorphism with temperatures between 500° to 700°C and pressures greater than about 2 kb.
- anoxygenic photosynthesis: the use of light to synthesize ATP without oxygen production. Reducing power is typically obtained from reduced substances such as Fe<sup>2+</sup>, H<sub>2</sub>S, H<sub>2</sub>, S<sub>2</sub>O<sub>3</sub><sup>2-</sup>, and S<sup>0</sup>.
- Archean: the oldest of the Precambrian Eons beginning with the earliest preserved rocks (about 4.0 Ga) and ending at 2.5 Ga.
- banded iron formation: laminated iron-rich sedimentary rocks, mostly from the Precambrian. Iron oxide and iron silicate-rich layers typically alternate with silica-rich layers.
- chemolithoautotroph: nonphotosynthetic organism that utilizes inorganic compounds as both electron acceptor and electron donor and that fixes CO<sub>2</sub> to build its organic biomass.
- chemoheterotroph: nonphotosynthetic organism that obtains all or part of its organic matter for biosynthesis from external sources.
- δ<sup>34</sup>S: the isotopic composition of sulfur species expressed as permil (parts per thousand) deviations from the Canyon Diablo Troilite (CDT) standard and given by:  $10^3 * [(^{34}\text{S}/^{32}\text{S})_{\text{sam}} - (^{34}\text{S}/^{32}\text{S})_{\text{CDT}}] / (^{34}\text{S}/^{32}\text{S})_{\text{CDT}}$
- dissimilatory: the breakdown of organic material to provide energy for cellular growth and metabolism.
- endosymbiont: the partner in a mutualistic relationship living inside another organism (the host).
- eukaryote: organisms of the Domain Eucarya containing a nucleus and in most cases other membrane-bound organelles.
- fermentation: energy production for cellular metabolism and growth where organic compounds act as both primary electron donor and ultimate electron acceptor.
- gene: a length of DNA encoding for a specific protein, structural RNAs, or various RNA molecules used in protein synthesis.
- genome: the complete set of genes in an organism.
- greenschist facies: metamorphic grade spanning a temperature range from around 250° to 450°C and burial pressures of between 1 to 8 kb.
- halophile: organisms of the Domain Archaea thriving in brines of high salinity.
- hyperthermophile: an organism with an optimal growth temperature of greater than 80°C.
- lateral gene transfer: the transfer of a gene from one organism to another.

- mesophile: an organism with an optimal growth temperature of between around 25° and 40°C.
- Mesoproterozoic: the middle of three Eras comprising the Proterozoic Eon beginning at 1.6 Ga and ending at 1.0 Ga.
- metabolism: the integrated set of biochemical reactions in a cell. Also refers to the principal means of energy gain used to fuel cell biochemistry.
- methanogen: an organism capable of producing methane. Pathways include the reduction of carbon dioxide with H<sub>2</sub>, and the fermentation of acetate.
- methanotroph: an organism capable of oxidizing methane.
- mineralization: the decomposition of organic material to its inorganic constituents.
- mutation: change in DNA composition. This results in alterations in the RNA transcribed from the DNA.
- Neoproterozoic: the youngest of three Eras comprising the Proterozoic Eon beginning at 1.0 Ga and ending at 0.544 Ga.
- oxygenic photosynthesis: the use of light to synthesize ATP with oxygen production.
- Paleoproterozoic: the oldest of three Eras comprising the Proterozoic Eon beginning at 2.5 Ga and ending at 1.6 Ga.
- photosystems I and II: coupled photosystems used to split water to form oxygen during oxygenic photosynthesis. A single photosystem acting alone provides the reducing power for anoxygenic photosynthesis (see definition) by green and purple bacteria.
- phototactic: movement towards light.
- phototroph: an organism obtaining energy from light.
- phylogeny: the evolutionary history of organisms.
- prokaryote: organisms of the Domains Bacteria and Archaea. They lack a nucleus and other membrane-enclosed organelles.
- Proterozoic: the youngest of the two Precambrian Eons spanning in age from 2.5 to 0.544 Ga.
- ribosome: small particles composed of RNA and protein, where protein synthesis takes place within a cell.
- thermophile: an organism with an optimal growth temperature of between around 45 and 70°C.
- zeolite facies: very low-grade metamorphism with maximum temperatures of around 250°C and maximum burial pressures of around 4 kbars.

## INTRODUCTION

It is likely that some of the earliest organisms on Earth gained energy from the metabolism of sulfur compounds (Stetter, 1996). Organismal evolution led to innovations in metabolic pathways, many of which certainly modified the surface chemistry of the Earth. Such changes in surface chemistry provided new metabolic opportunities that promoted further evolutionary innovations. For example, anoxygenic photosynthesis may have provided the sulfate necessary to promote the evolution of sulfate-reducing bacteria (Schidlowski, 1989). Furthermore, the evolution of oxygenic photosynthesis provided for diverse metabolic possibilities including nitrification, methanotrophy, and non-photosynthetic sulfide oxidation. Some of these relationships have been inferred from stable isotope studies and logical deduction (Hayes, Kaplan, and Wedeking, 1983; Hayes, Lambert, and Strauss, 1992; Schidlowski, 1989). A much deeper understanding of the evolutionary relationship among organisms, however, is possible from phylogenies derived from comparisons of the small subunit (SSU) of the ribosomal RNA molecule (rRNA) comprising the 16S subunit for prokaryotic organisms and 18S subunit for eukaryotes (Woese, 1987). These phylogenies have become an integral part of the discussions of the evolutionary relationships among organisms for several years (Woese and Fox, 1977; Woese, 1987; Stetter, 1996; Sogin and others, 1996).

The most complete understanding of the course of the chemical evolution of the surface environment will fully integrate organismal phylogenies with geochemical and geological evidence for surface change. In this way the influence of biological evolution on Earth-surface change, and vice versa, the influence of such changes on subsequent biological evolution, can be fully appreciated. These discussions have already begun (Knoll and Bauld, 1989; Schopf, 1996; Knoll and Canfield, 1998), but a full integration of phylogenetic and geological information has yet to be realized. We offer this manuscript as a small further step in this direction. Herein we will consider the evolutionary relationships among the organisms of the sulfur cycle based mostly on SSU rRNA phylogenies, and we will integrate this with geological evidence of Earth-surface change.

#### BIOLOGICAL EVOLUTION

The comparative analysis of SSU rRNA sequences to infer phylogenetic relationships among organisms has produced the first real metric by which bacterial evolution may be ordered (Woese, 1985). As a result, every bacterium now has an evolutionary history and hence a story to tell; this has given a much expanded significance to the field of microbial ecology. The premises behind using the SSU rRNA molecule are as follows: (1) the function of the ribosome, that is protein synthesis, is constant among all organisms, (2) the small subunit of the rRNA molecule is universal in distribution, (3) the small subunit is large, containing approx 1500 nucleotides in prokaryotes and 2300 nucleotides in eukaryotes, providing a large information content, and (4) the SSU rRNA subunit is moderately to highly conserved across great phylogenetic distances (Woese, 1987), allowing sequences from widely different forms of life such as, for example, methanogenic bacteria and butterflies, to be compared.

Over time, mutations accumulate in the ribosomal RNA molecule. In the SSU rRNA subunit mutations accumulate only slowly, which is one reason why this molecule has proven to be so useful in comparing evolutionary relationships among widely disparate organisms. The mutations that accumulate are functionally neutral, meaning that they do not affect the function of the ribosome (for the survival of the organism only a limited change in the function of the ribosome can be accepted). For the most part, mutations within the SSU rRNA molecule are random, although in some regions of the molecule coupled changes may also occur, and these regions are generally not used for phylogenetic analysis (Olsen and Woese, 1993). A basic assumption behind constructing phylogenies using SSU rRNA is that the SSU rRNA of all modern organisms derived ultimately from a common ancestor (or ancestors with similar genomes; Woese, 1998). In this way, all organisms contain some memory of their ancient history, and this history is reconstructed through comparative sequence analysis (Woese, 1987).

Another basic assumption in re-creating evolutionary relationships based on SSU rRNA is that the evolutionary history of the SSU rRNA reflects the evolutionary history of the organism. This will be true if, as commonly postulated, “. . . a clear majority of the essential genes in a genome share a common heritage” (Olsen and Woese, 1993). This requires, in turn, that lateral transfer of genetic material from one organism to another has not been the predominant means by which the genome was established. The fact that a number of essential genes provide similar reconstructions of the evolutionary history of organisms suggests that organisms do, generally, have a phylogeny and a characteristic evolutionary history (Olsen and Woese, 1993; Wagner and others, 1998). At least some lateral gene transfer, however, does occur (Rudi, Skulberg, and Jakobsen, 1998; Woese, 1998), and the full significance of this for organismal phylogenies will become more apparent when a large number of whole genome sequences become available (Olsen and Woese, 1993). Preliminary indications from the small number of available whole genome sequences are equivocal providing evidence both for and against lateral gene

transfer as an important mechanism of gene acquisition in prokaryotes (Nelson and others, 1999; Snel, Bork, and Huynen, 1999).

*The tree of life.*—Sequence analysis of SSU rRNA has revealed that all life can be divided into three principal Domains (fig. 1; Olsen and Woese, 1993); these are the Bacteria, the Archaea, and the Eucarya. The stem of the tree, denoting the last common ancestor, divides the Bacteria and the Archaea; in this way the Eucarya and Archaea are more closely related to each other than either of these two Domains are to the Bacteria. The rooting of the tree comes not from SSU rRNA analysis but rather from the analysis of gene duplicating events that preceded the emergence of the three Domains (Gogarten and others, 1989; Iwabe and others, 1989).

The SSU rRNA-based tree of life bears little resemblance to the “classic” tree of life which divided the living world into five Kingdoms (Whittaker and Margulis, 1978): Animalia, Plantae, Fungi, Protista, and Monera (prokaryotes). The new tree of life emphasizes the genetic diversity of prokaryotes (Bacteria and Archaea) and shows that the history of life on Earth is largely a history of prokaryotic evolution, not the evolution of macroscopic organisms as previous phylogenetic schemes would suggest. In fact, three of the previous dominant Kingdoms: Animalia, Plantae, Fungi, are found to occupy crown positions within the Domain Eucarya, with little diversity in SSU rRNA sequences to separate them.

In what follows, we will offer a brief tour through the tree of life (fig. 1), focusing on the prokaryotic organisms (Domain Archaea and Domain Bacteria) as the phylogenies of these organisms depict the most important historical developments in sulfur metabolism. Our interpretations are founded on the current SSU rRNA-based tree of life and may need to be modified if and when more compelling phylogenies become available.

*Domain Archaea.*—The Archaea are prokaryotic organisms since they, like the Bacteria (see below), lack a nucleus and contain no organelles. They are also distinctive from the Bacteria in many ways, including the universal presence of unique, branch-chain, ether-linked membrane lipids, and regions of unique sequence signature in the SSU rRNA molecule (Woese, 1987). It has normally been thought that the Archaea occupy environmental extremes of temperature (hyperthermophiles), salinity (halophiles), and redox potential (methanogens) (Woese, 1987). A much wider ecological role for Archaea is indicated, however, by the recent identification of Archaea as an important constituent of marine picoplankton (DeLong, 1992; DeLong and others, 1994) and the presence of Archaea in symbiotic association with a species of Axinellid sponge (Preston and others, 1996). None of these organisms has yet been cultured, so their physiologies are currently unknown.

The Domain Archaea is comprised of three Kingdoms: the Crenarchaeota, the Euryarchaeota, and the Korarchaeota. While the Crenarchaeota and the Euryarchaeota have members with well-studied physiologies, the Korarchaeota are known only from molecular sequences and are not therefore shown in figure 1. A large range of metabolisms is found among organisms within the Euryarchaeota; these include methanogenesis, elemental sulfur reduction, fermentation, sulfate reduction, aerobic respiration, and a unique kind of photosynthesis among some members of the halophiles that relies on the protein bacteriorhodopsin. This protein is functionally and structurally similar to the eye pigment rhodopsin. The deepest branches within the Euryarchaeota house hyperthermophilic organisms with maximum growth temperatures of up to 110°C (Stetter, 1996). These include members of the genus *Methanopyrus* (methanogens) and *Pyrococcus* (sulfur reducers).

The Crenarchaeota is dominated by hyperthermophilic organisms, many of which gain energy by the metabolism of elemental sulfur (see below). The Korarchaeota (not shown) branch from near the base of the Crenarchaeota (Barns and others, 1996). Like most of the deep-branching organisms of the Euryarchaeota and Crenarchaeota, the

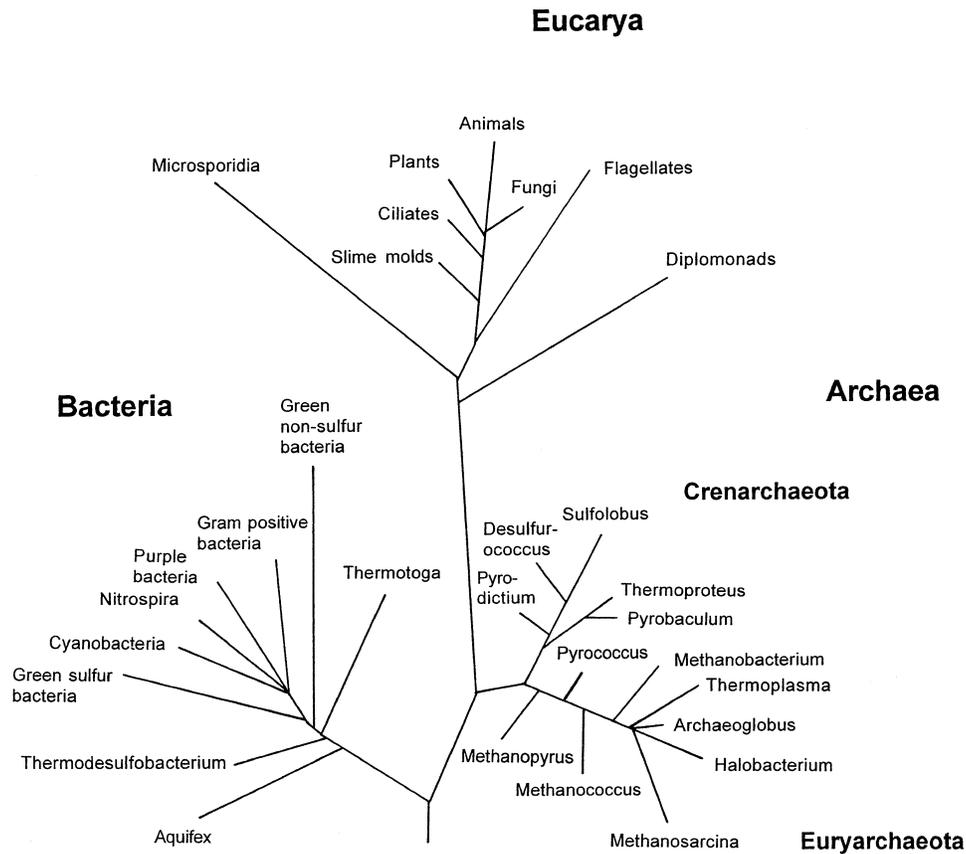


Fig. 1. This figure shows several of the principal lineages within the Tree of Life determined from the comparison of SSU rRNA sequences. Within the Bacteria and Archaea, the lineages represented are mainly those having some relevance to the evolution of the sulfur cycle. This figure is abstracted from the phylogenetic trees presented in Olsen and Woese (1993), Stetter (1996), and Pace (1997). See text for details on the construction and interpretation of this phylogenetic tree.

Korarchaeota also have a hyperthermophilic association (Barns and others, 1996). For both the Crenarchaeota and Euryarchaeota the oxidation of molecular hydrogen is a common metabolism.

Most deep-branching organisms within the Archaea are hyperthermophilic, and the Archaea are themselves less derived (shorter SSU rRNA sequence distance) than the other Domains relative to the last common ancestor. These observations have led to speculations that the common ancestor was also hyperthermophilic (Woese, 1987; Stetter, 1996). This hypothesis has not been universally accepted (Galtier, Tourasse, and Gouy, 1999) but, nonetheless, has fueled further debate as to the possibility that life itself has originated in a high temperature environment (Russell and Hall, 1997; Shock, 1996).

*Domain Bacteria.*—The Domain Bacteria houses most of the prokaryotes with which we are most familiar. These organisms conduct an enormous range of metabolisms including: fermentation, acetogenesis, sulfate reduction, elemental sulfur reduction, metal oxide reduction, denitrification, nitrification, aerobic respiration, oxygenic and anoxygenic photosynthesis, and the whole range of chemolithoautotrophic metabolisms using oxygen, nitrate, and possibly metal oxides, as electron acceptors. Like the Archaea,

the deepest-branching lineages within the Bacteria house hyperthermophilic organisms; these include the chemolithoautotrophic Aquifex-Hydrogenobacter group, sulfate reducers of the genus *Thermodesulfobacterium*, and the dominantly fermentative organisms of the Thermotogales group (Stetter, 1996; Pace, 1997). The fact that deep-branching organisms within the Domain Bacteria are hyperthermophilic lends further support to the idea that the last common ancestor was also hyperthermophilic.

Branching a little farther up tree from the hyperthermophilic Thermotogales and *Thermodesulfobacterium* groups are the green non-sulfur bacteria which includes several anoxygenic phototrophs, of which *Chloroflexus aurantiacus* is a well-known example (Pierson and Castenholz, 1992). These phototrophs are the deepest-branching photosynthetic organisms, and oxidize, via a single photosystem, hydrogen sulfide to elemental sulfur and sulfate. Farther up are found the green sulfur bacteria, which contain numerous anoxygenic phototrophs of the family Chlorobiaceae.

Farther up still, the tree indicates a tremendous radiation of bacterial life. From this radiation emerges most of the Bacterial Domain. Some of the more conspicuous groups include the cyanobacteria (oxygenic phototrophs), the purple bacteria (an enormous variety of heterotrophic, chemolithoautotrophic, and anoxygenic phototrophic bacterial types), and the gram-positive bacteria (including a wide variety of anoxygenic phototrophs, fermentative bacteria, and heterotrophic bacteria, including many thermophilic organisms).

#### BACTERIA OF THE SULFUR CYCLE

The ability to metabolize sulfur compounds is widespread throughout the Domains Bacteria and Archaea. We will use the SSU rRNA-based molecular phylogenies to provide an indication of the order of emergence of the principal sulfur metabolisms. In most cases the sulfur-based metabolism within the indicated lineage can be traced to near the bottom of the lineage. We believe, therefore, the branch point of the lineage on the main stem of the tree provides some indication of the evolutionary depth of the metabolism. However, the history of metabolic innovations provided by this comparison is only as good as the branching patterns and evolutionary depths provided by the SSU rRNA molecular phylogeny. We have discussed earlier some of the assumptions that are made when using SSU rRNA phylogenies to infer organismal evolution, and these points will be re-examined where appropriate.

*Elemental sulfur reduction.*—The reduction of elemental sulfur to hydrogen sulfide, utilizing both H<sub>2</sub> and organic compounds as the electron donor, is widespread within both the Domains Archaea and Bacteria (fig. 2). Significantly, elemental sulfur reduction is found among the most deeply-branching hyperthermophiles in the Archaea (Stetter, 1990; Stetter, 1996). Likewise, hyperthermophiles of the Order Thermotogales, within the Bacterial Domain (fig. 2), are also capable of reducing elemental sulfur (Schauder and Kröger, 1993). The deep branching of elemental-sulfur reduction in both of the prokaryotic Domains suggests that this metabolism is the most ancient of those utilizing sulfur (Stetter and Gaag, 1983); it may also rank among the most primitive of the early metabolisms (Stetter and Gaag, 1983). The hyperthermophilic nature of deep-branching elemental-sulfur reducers suggests that this metabolism may have evolved in a high temperature environment (Stetter, 1990).

A plausible pathway for elemental sulfur formation in an early anoxic atmosphere is the reaction between mantle-derived hydrogen sulfide and sulfur dioxide gas emanating from volcanic fumarols. Hydrous fluids derived from relatively oxidized magmas may be rich in both sulfur dioxide and hydrogen sulfide (Hattori and Cameron, 1986), and the rapid cooling of these volcanic gases shifts the equilibrium in favor of elemental sulfur formation (eq 1; Grinenko and Thode, 1970).



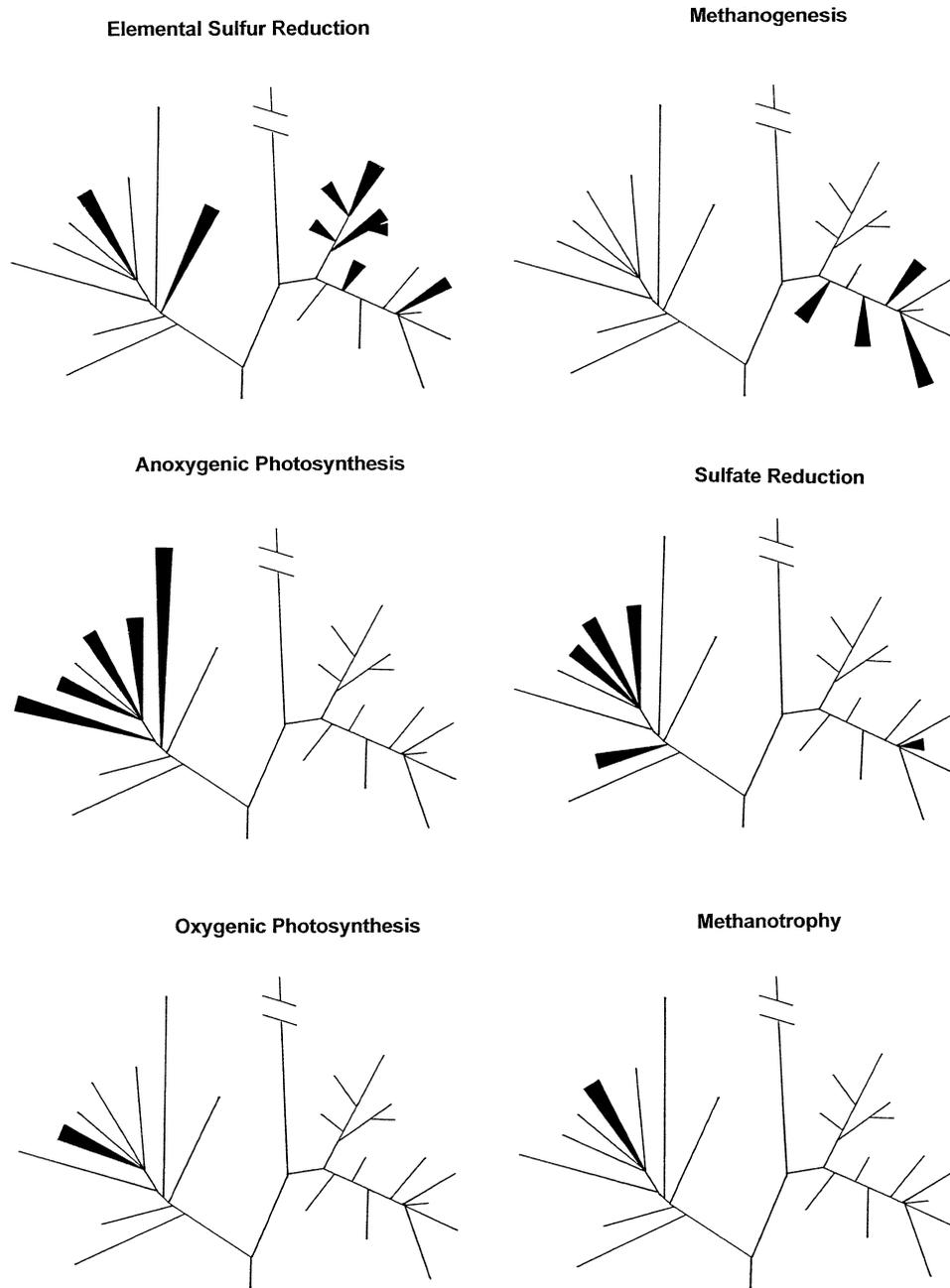


Fig. 2. This figure depicts the lineages supporting key metabolisms of the sulfur cycle, as well as other important metabolisms with relevance for the cycling of sulfur. The Archaeal and Bacterial Domains are represented here, while the Domain Eucarya is not shown. See figure 1 as a reference for the lineages depicted.

Terrestrial volcanic fields such as found in the North Island of New Zealand, Yellowstone Park, and Iceland may be viewed as possible analogs of early ecosystems supporting elemental sulfur reduction.

*Anoxygenic photosynthesis.*—The capacity to conduct anoxygenic photosynthesis is widespread among the Bacteria (Woese, 1987; fig. 2). The deep branching of both the Green non-sulfur bacteria and the Green sulfur bacteria indicates that anoxygenic photosynthesis is ancient (Woese, 1987). This is further indicated by the use of a single photosystem to harvest energy from the sun, a process far simpler than the coupled photosystems I and II required for oxygenic photosynthesis (Blankenship, 1992). Therefore, it is not surprising that some of the lineages supporting anoxygenic photosynthesis branch much deeper than the cyanobacterial lineage where oxygenic photosynthesis is solely housed (Woese, 1987; fig. 2). It is interesting, however, that the largest radiation of anoxygenic phototrophs is apparently associated with, at least within the resolution of SSU rRNA phylogenies, the development of oxygenic photosynthesis. It is plausible that the ability to fix carbon by the splitting of the ubiquitous water molecules (oxygenic photosynthesis) provided abundant organic material that allowed, during subsequent oxidation, the production of large amounts of chemically reduced compounds like  $H_2S$  and  $Fe^{2+}$  (Widdel and others, 1993). These can serve as electron donors during anoxygenic photosynthesis. A large increase in the environments containing possible electron donors could have set the stage for an evolutionary radiation of anoxygenic photosynthetic organisms.

*Sulfate reduction.*—An important geological consequence of anoxygenic photosynthesis is the production of sulfate which can further be used as an electron acceptor in the remineralization of organic carbon by sulfate-reducing bacteria (Schidlowski, 1989). For this reason it may not be surprising that the deepest-branching sulfate-reducing bacteria branch is nearly as deep as the deepest-branching anoxygenic photosynthetic bacteria (fig. 2). It may be of some significance that the anoxygenic phototroph *Chloroflexus aurantiacus* (green non-sulfur bacteria) is thermophilic, withstanding temperatures of up to 70°C (Pierson and Castenholz, 1992), while the deepest branching sulfate reducers (*Thermodesulfobacterium* sp.) are hyperthermophilic, with temperature maxima of 85°C (Stackebrandt, Stahl, and Devereux, 1995). Both hyperthermophilic sulfate reducers and *Chloroflexus* have been isolated from modern hot springs containing a significant source of primary reduced sulfur (Zeikus and others, 1983; Pierson and Castenholz, 1992). These modern environments might resemble ancient settings in which hyperthermophilic sulfate reducers and the earliest anoxygenic phototrophs evolved and cohabited.

Hyperthermophilic sulfate reducers are also found among the Archaea and are represented by several species within the genus *Archaeoglobus* (Stetter, 1992; 1996). *Archaeoglobus* does not branch particularly deeply within the Archaea and is located, rather conspicuously, among a group of methanogenic bacteria and shares many common enzymes and biochemical pathways with the methanogens (Stetter, 1995). This has led to speculations that *Archaeoglobus* obtained the ability to reduce sulfate by lateral gene transfer of the sulfate-reduction apparatus (Stetter, 1996). If so, the event was apparently quite ancient, as similar phylogenies are derived from sequence comparisons of both the sulfite-reductase gene and the SSU rRNA molecule (Wagner and others, 1998). The sulfite-reductase gene encodes for one of the primary enzymes within the sulfate-reduction process.

A large radiation of sulfate-reducing bacteria accompanies the general radiation of bacterial life (fig. 2). The largest population of sulfate-reducing bacteria is found within the  $\delta$ -subdivision of the purple bacteria, and most are mesophilic organisms (Widdel and Bak, 1992). Diverse sulfate-reducing bacteria are also found among the gram-positive bacteria (Widdel, 1992), many of which are thermophilic. A lone thermophilic species

has been isolated from the Nitrospira group, which also apparently originates within the same general radiation that produced the cyanobacteria, the gram-positive bacteria, and the purple bacteria, among other important bacterial lineages (Pace, 1997).

*Other bacteria of the sulfur cycle.*—Numerous other bacterial types are involved in the cycling of sulfur. For example, sulfur compounds of intermediate oxidation state (elemental sulfur, thiosulfate, sulfite) can be actively disproportionated (Bak and Pfennig, 1987; Thamdrup and others, 1993; Fuseler and others, 1996), and some organisms use disproportionation reactions as their sole means of energy conservation (Finster, Liesack, and Thamdrup, 1998). At present, all of the bacteria known to disproportionate sulfur compounds of intermediate oxidation state are found within the  $\delta$ -subdivision of the purple bacteria.

The ability of organisms to oxidize sulfur compounds chemolithoautotrophically is widespread among the two prokaryotic Domains. Among the hyperthermophiles, deep-branching members of the Aquifex/Hydrogenobacter group in the Bacterial Domain can oxidize hydrogen sulfide using  $O_2$  (Stetter, 1996) as can several known members of the Archaeal Domain (Stetter, 1996). Aquifex/Hydrogenobacter is a rather strange group of organisms, as its microaerophilic habitat is rather inconsistent with its deep-branching placement. In general, deeper-branching lineages house anaerobic organisms while aerobic organisms tend to occupy higher positions on the tree (Woese, 1987), reinforcing well accepted notions (Oparin, 1957) that life originated in an anaerobic environment. It has been suggested that the aerobic lifestyle of Aquifex/Hydrogenobacter developed over the course of evolution (Fenchel and Finlay, 1995) and does not represent the ancestral metabolism of this group. Other suggestions for the enigmatic metabolism of Aquifex/Hydrogenobacter include an incorrect phylogenetic position from the SSU rRNA sequences (Brown and Doolittle, 1997) and the evolution of aerobiosis at the extremely low partial pressure of oxygen that the photodissociation of water would provide (Decker and others, 1998).

Of considerable significance for the process of sulfide oxidation is the large radiation of sulfide-oxidizing bacteria emerging from the base of the  $\alpha$ - and  $\beta$ -subdivisions of the purple bacteria (Muyzer and others, 1995). Most known non-photosynthetic, sulfide-oxidizing bacteria are found within these two subdivisions, and this radiation is, therefore, of some evolutionary significance. It has been proposed that evolutionary radiations of organisms are promoted by profound environmental changes (Woese, 1987). If so, an increase in the availability of oxygen within the environment might have been the driving mechanism for the radiation of non-photosynthetic sulfide-oxidizing bacteria (Canfield and Teske, 1996). Using a geologically calibrated SSU rRNA sequence divergence rate of sulfide-oxidizing endosymbionts, Canfield and Teske (1996) have suggested that sulfide-oxidizing bacteria of the  $\alpha$ - and  $\beta$ -subdivisions of the purple bacteria emerged between 0.75 to 1 BY ago.

*Summary.*—From the SSU rRNA phylogeny, we can envision early Earth ecosystems with well developed sulfur cycles thriving in environments receiving reduced sulfur gases (and  $H_2$ ) from the mantle. Such environments might include recirculating hydrothermal systems as found in areas of ocean crust formation (spreading centers) and places of active terrestrial volcanism. These environments were likely thermophilic to hyperthermophilic with primary production occurring by anoxygenic photosynthesis when light was available. In the absence of light, organic matter production occurred by chemolithoautotrophic metabolisms utilizing  $H_2$  as the electron donor and oxidized species such as elemental sulfur (forming  $H_2S$ ) or carbon dioxide (forming methane) as the electron acceptors. Organic compounds were oxidized by the reduction of elemental sulfur and sulfate (chemoheterotrophic metabolisms), as well as by the fermentation of acetate, producing methane. Elemental sulfur was produced by the reaction between  $H_2S$  and

SO<sub>2</sub>, while anoxygenic photosynthesis was an important source of sulfate. The evolution of oxygenic photosynthesis produced a dramatic increase in the primary production of organic material and promoted a profound expansion of the ecosystems available to prokaryotic life. This increase in ecosystem space provided for a dramatic evolution of new bacterial life including most of the bacteria of the sulfur cycle. Much later, probably in the late Proterozoic, increasing oxygen levels spurred a radiation of numerous lineages of non-photosynthetic, sulfide-oxidizing bacteria.

#### GEOLOGICAL EVOLUTION

An ultimate goal is to link evolutionary events as deduced from the SSU rRNA phylogenies to specific evidence for environmental change in the geologic record. To do so accurately, we must faithfully reconstruct the history of environmental change, and we will focus considerable attention on this issue. Our emphasis will be on the history of the sulfur cycle and any specific evidence for changes in the cycling of sulfur. We will highlight where the geologic record may provide evidence for the evolution of biological metabolisms. In the present context, indicators of environmental change will come mainly from the history of stable sulfur isotopes and ancient shale geochemistry, including the abundance of iron, sulfur, and carbon species.

*Stable isotopes.*—The isotopic composition of sedimentary sulfides provides primary information on the evolution of the sulfur cycle (Schidlowski, Hayes, and Kaplan, 1983; Hayes, Lambert, and Strauss, 1992; Canfield, 1998). Sulfate-reducing bacteria preferentially reduce  $^{32}\text{SO}_4^{2-}$ , thereby producing sulfides depleted in  $^{34}\text{S}$  (Harrison and Thode, 1958; Kaplan and Rittenberg, 1964). The systematics governing the fractionation of isotopes by sulfate-reducing bacteria and other aspects of the sulfur cycle relevant to the isotopic composition of sedimentary sulfides have recently been reviewed by Knoll and Canfield (1998). A synopsis of the important factors controlling the isotopic composition of sedimentary sulfides is presented table 1.

A view of the isotopic composition of sedimentary sulfides and seawater sulfate, through geologic time, is presented in figure 3. The sulfides included in this compilation are from sedimentary rocks (mostly shales) and generally associate with organic carbon. They, therefore, have the highest probability of a biological origin. Sulfides from heavily mineralized regions of probable hydrothermal origin and of high metamorphic grade have not been included (an exception is the amphibolite-grade rocks from Isua, Greenland). The isotopic record of seawater sulfate, as represented by evaporitic sulfate minerals, is spotty through most of the Precambrian and provides an important, albeit incomplete, reference point for consideration of sedimentary sulfide isotopic compositions.

*Archean.*—Through most of the Archean the isotopic composition of sedimentary sulfides cluster around the mantle value of near 0 permil, with a total spread of approx 10 permil. This variability has been interpreted to indicate that dissimilatory sulfate reduction was responsible for sulfide formation, even in sulfides as old as those from Barberton, South Africa at 3.4 Ga (Ohmoto, Kakegawa, and Lowe, 1993). Although poorly studied, the isotopic fractionation associated with assimilatory sulfate reduction, the incorporation of sulfate into the organic sulfur compounds of living organisms, may be >5 permil (Chambers and Trudinger, 1979). Also, the isotopic composition of non-biological magma-derived sulfides may range to  $\pm 4$ –5 permil of the mantle value (Schneider, 1970). These isotopic variations are similar to those observed in early Archean sediments, and, therefore, the available isotopic data alone do not unambiguously implicate the activities of sulfate reducing bacteria.

If sulfate reduction was responsible for sulfide formation in the Archean, then, the generally small observed fractionations would suggest sulfate-limiting conditions. This

TABLE 1

*Considerations for interpreting the isotopic composition of sedimentary sulfides*

1. With non-limiting sulfate, pure cultures of sulfate reducing bacteria produce a wide range of fractionations of between 4 to 46 permil (4, 9, 10).
2. Isotope fractionation during sulfate reduction is governed, albeit imprecisely, by the specific rate of sulfate reduction; that is, the rate of sulfate reduction per bacterial cell (9). The highest fractionations are found at the lowest specific rates of sulfate reduction (5).
3. Reported fractionations with H<sub>2</sub> as an electron donor range from 3 to 16 permil (9, 10).
4. Fractionations of <4 permil have been reported when sulfate levels drop below around 1 mM (8). At higher concentrations, sulfate becomes non-limiting, and for natural populations of sulfate reducing bacteria, we have found fractionations of 28 per mil with 1.5 mM sulfate (11).
5. Natural populations of sulfate reducing bacteria, with non-limiting sulfate, produce fractionations between 16 permil to 42 permil (6). Consistently high fractionations suggest that H<sub>2</sub> may not be an important substrate for sulfate reduction in natural populations. As with pure cultures, there is some evidence for control of isotope fractionation by the specific rates of sulfate reduction (6). Sulfides more depleted in <sup>34</sup>S than 45 permil compared to sulfate have not been reported as a product of sulfate reduction with either natural or pure cultures of sulfate reducing bacteria.
6. Depletions of sulfide in <sup>34</sup>S of greater than 45 permil, and up to 70 permil, as found commonly in marine sediments, cannot be explained by the activities of sulfate reducing bacteria alone (1, 2). The disproportionation of oxidized sulfur intermediates including S<sup>0</sup>, S<sub>2</sub>O<sub>3</sub><sup>2-</sup> and SO<sub>3</sub><sup>2-</sup>, produces isotopically-depleted sulfides, and this process, operating through several cycles, can explain the large <sup>34</sup>S-depletions in sedimentary sulfides (1, 2, 3, 7).
7. Due to the isotopic fractionation associated with sulfate reduction, sulfate becomes progressively enriched in <sup>34</sup>S as it becomes consumed with depth in sediments. The sulfide produced with depth also becomes enriched in <sup>34</sup>S. Sulfate reduction rates are most rapid near the sediment surface and most pyrite forms here. This is also a region where sulfate depletion is minimal at modern marine seawater sulfate concentrations. Therefore, the influence of sulfate depletion on the isotopic composition of sedimentary sulfides is not large (12). This effect, however, may become more pronounced as sulfate levels drop and a higher proportion of solid phase sulfides form in the sediment region experiencing significant sulfate depletion.

References: (1) Canfield and Thamdrup, 1994; (2) Canfield and Teske, 1996; (3) Canfield, Thamdrup, and Fleischer, 1998; (4) Chambers and others, 1975; (5) Chambers and Trudinger, 1979; (6) Habicht and Canfield, 1997; (7) Habicht, Canfield, and Rethmeier, 1998; (8) Harrison and Thode, 1958; (9) Kaplan and Rittenberg, 1964; (10) Kemp and Thode, 1968; (11) Canfield, unpublished results; (12) Knoll and Canfield, 1998.

would require sulfate levels <1 mM, given what we presently know about the influence of sulfate concentration on isotope fractionation (table 1).

Some Archean environments experienced at least locally elevated concentrations of sulfate as evidenced by the precipitation of evaporitic sulfate minerals. A well-studied example is the 3.5 Ga chert-barite unit from the Warrawoona Group, Western Australia (Buick and Dunlop, 1990). The shapes of the precipitated barite (BaSO<sub>4</sub>) crystals indicate that the sulfate was initially precipitated as gypsum (CaSO<sub>4</sub> · 2H<sub>2</sub>O). These deposits formed in a variety of shallow water and shoreline settings within a volcanic terrain that was apparently barred from the open ocean by stranded pumice rafts. Microlaminae of pyrite that precipitated synchronously with the sulfates are associated with some of the barites (Buick and Dunlop, 1990). The isotopic composition of the sulfur in the barites averages about 3.6 permil, while the isotopic composition of the associated pyrite sulfur averages near 0 permil (Lambert and others, 1978). A biologically and geologically consistent explanation for the origin of the sulfate is the anoxygenic phototrophic oxidation of primary mantle-derived sulfide to sulfate.

Another possible sulfate source might have been the hydrolysis of volcanogenic SO<sub>2</sub> originating from relatively oxidized magmas (eq 2; Hattori and Cameron, 1986). Whether from biological or volcanogenic sources, the evaporitic sulfate may be locally derived and need not have been precipitated from seawater with high sulfate concentrations. Isotope fractionation accompanies SO<sub>2</sub> hydrolysis and magmatically-derived sulfate is typically 7 to 12 permil enriched in <sup>34</sup>S compared to sulfide (Hattori and Cameron, 1986). Therefore, an enrichment of evaporitic sulfate in <sup>34</sup>S by 7 to 12 permil compared to sulfide indicates SO<sub>2</sub> hydrolysis as a principal pathway of sulfate formation.

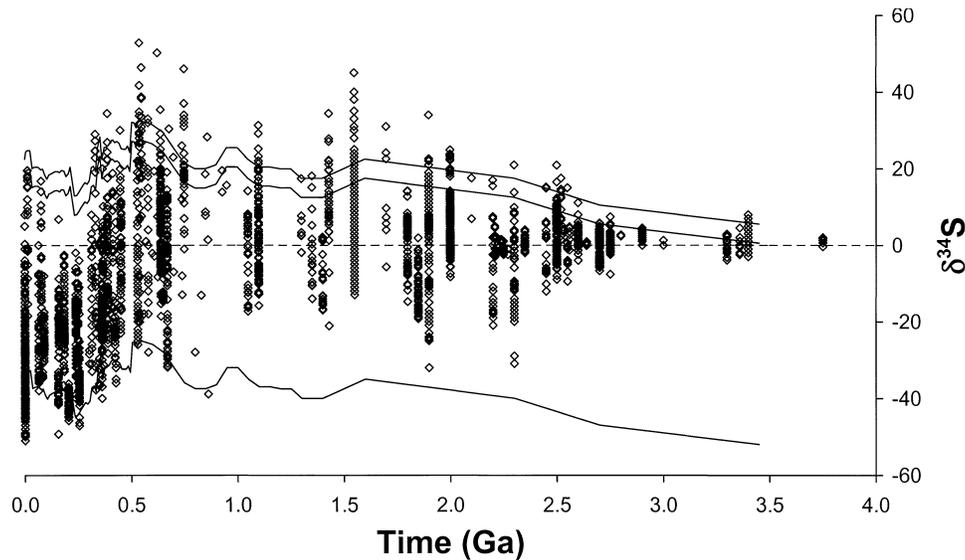
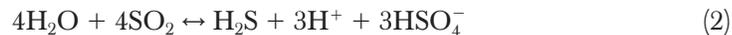


Fig. 3. The isotopic composition of sedimentary sulfides over geologic time is shown. Only sulfides of probable biological origin are included in this figure; therefore sulfides from sediments from hydrothermal areas, from highly mineralized areas, and of high metamorphic grade have not been included (see text). For comparison, the isotopic composition of seawater sulfate, as determined from evaporitic sulfate minerals, is shown as a band with a width of 5 permil. This compilation is produced from the supplemental information provided with Canfield (1998) plus additional data from Carrigan and Cameron (1991), Logan and others (1999), Melezhik, Grinenko and Fallick (1998), and Kakagawa, Kawai and Ohmoto (1999). Ages for sediments of the Transvaal Supergroup have been modified from earlier compilations of Canfield (1998) and Knoll and Canfield (1998) with new, modern, chronologies (for a summary of chronological constraints see Buick and others, 1998). The new ages are generally older than previous determinations. A complete list of data and references are available on request.

This was apparently the case in many Archean volcanic settings (Hattori and Cameron, 1986).



The small enrichment of  $^{34}\text{S}$  in the sulfate from the Warrawoona Group is consistent with the small fractionations that accompany both anoxygenic photosynthesis (Fry, Gest, and Hayes, 1984), and the precipitation of sulfate minerals from solution (Claypool and others, 1980). If dissimilatory sulfate reduction had been active in such a sulfate-enriched basin, then large fractionations, similar to those found from modern sulfate-reducing bacteria, would be expected. There are no reports of such highly  $^{34}\text{S}$ -depleted sulfides from the basin. The discovery of highly fractionated sulfides in such ancient evaporitic settings would lend considerable support to the existence of sulfate-reducing bacteria at this time.

By contrast with the chert-barite deposits from Warrawoona, the 2.7-Ga Hemlo gold deposit of the Superior Province of Canada houses massive sulfate deposits and has associated sulfides with  $\delta^{34}\text{S}$  values as low as  $-17.5$  permil (Cameron and Hattori, 1987). Nearly all of the highly  $^{34}\text{S}$ -depleted sulfides are associated with hydrothermal mineralization, and many clearly have a hydrothermal origin (Cameron and Hattori, 1987). In other instances, the sulfides may be of biological origin, and if so, this would be the first clear isotope evidence for dissimilatory sulfate reduction (Goodwin, Monster, and

Thode, 1976; Thode and Goodwin, 1983). For shales of similar age and unassociated with mineralization, the isotopic compositions of sulfides are within 5 to 10 permil of the mantle value (Cameron and Hattori, 1987; Strauss, 1986). Thus, it is likely that elevated sulfate concentrations, in what was probably a closed basin, provided the opportunity for isotope fractionation to be expressed at Hemlo (Cameron and Hattori, 1987) whether by inorganic or biological reduction.

*Proterozoic.*—By 2.5 Ga, the isotopic composition of sedimentary sulfides commonly spans to  $\delta^{34}\text{S}$  values of  $-8$  to  $-10$  permil (Schidlowski, Hayes, and Kaplan, 1983; Hayes, Lambert, and Strauss, 1992; Knoll and Canfield, 1998; fig. 3). The isotopic composition of sulfate at 2.5 Ga is uncertain and rather poorly constrained between  $\delta^{34}\text{S}$  values of 8 permil for anhydrite and barite from 2.7 Ga mineralized sediments from the Superior Province of Canada and 15 permil for sediment-hosted anhydrite from the Huronian Supergroup in Canada. The difference in isotopic composition between sedimentary sulfides and seawater sulfate are at maximum around 15 to 20 permil at 2.5 Ga. These are significant fractionations and indicate sulfide formation by sulfate reduction. They are, however, less than the large fractionations of around 40 to 45 permil that sulfate-reducing bacteria can produce with non-limiting sulfate. The available experimental results suggest that reduced fractionations occur at sulfate levels  $<1$  mM (table 1), and our own results on natural populations show high fractionations of  $>28$  permil during sulfate reduction with  $>1.5$  mM sulfate. Moderate isotope fractionations during sulfate reduction indicate sulfate concentrations in the range of 1 mM at 2.5 Ga.

It is not until around 2.3 Ga that the large fractionations typical for sulfate-reducing bacteria with non-limiting sulfate (up to 40–45 permil, fig. 3) are first observed. The isotopic data support the conclusion that non-limiting sulfate concentrations  $>1$  mM first accumulated into the oceans during the early Proterozoic (see also Hayes, Lambert, and Strauss, 1992; Cameron, 1982; Knoll and Canfield, 1998). The isotope data does not, however, put upper limits on sulfate concentrations. Other lines of evidence will help constrain sulfate levels between around 1.8 and 2.3 Ga (see below).

Large fractionations of up to 40 to 45 permil between seawater sulfate and sedimentary sulfides are a continuous feature through the remainder of the Proterozoic, until the Neoproterozoic, where a further jump in fractionations to 55 permil or greater is observed (fig. 3). The timing of this jump in fractionations is uncertain and is bracketed somewhere between 0.64 to 1.05 Ga (Canfield and Teske, 1996; fig. 3). These large fractionations exceed those characteristic of sulfate-reducing bacteria acting alone and indicate additional fractionations due to the disproportionation of sulfur intermediate compounds (Canfield and Thamdrup, 1994; table 1). Canfield and Teske (1996) have argued that the expression of the oxidative part of the sulfur cycle was made possible by increasing atmospheric oxygen levels to  $>10$  percent of present levels. This level of oxygen was sufficient to oxidize the surface of coastal marine sediments, allowing for the efficient oxidation of sedimentary sulfides. As previously mentioned, sequence divergence in the SSU rRNAs of non-photosynthetic sulfide-oxidizing bacteria were calibrated and indicate that this bacterial group evolved also in the Neoproterozoic. This evolutionary event may have been promoted by an increase in oxygen levels at this time (Canfield and Teske, 1996).

*Banded iron formations.*—Iron-rich sediments, banded iron formations (BIFs), are a persistent part of the sedimentary record through the Archean and into the middle Proterozoic (up to 1.8 Ga). The great lateral extent of individual BIF formations and the wide distribution of BIFs in space and time has led to the generally accepted notion that BIFs precipitated from an ocean with iron-rich deep water (Holland, 1984; Klein and Beukes, 1993). Continuous deep-ocean anoxia, as is required to explain BIF formation, argues for levels of atmospheric oxygen lower than today's (Holland, 1984; Canfield, 1998). The discovery of anoxygenic phototrophs capable of oxidizing ferrous to ferric

iron demonstrates that BIFs could, in fact, have formed in the complete absence of oxygen (Widdel and others, 1993). Low levels of atmospheric oxygen, particularly in the Archean, are supported by other lines of geochemical evidence including the loss of Fe from well-developed soil profiles and the persistence of redox-sensitive minerals in fluvial deposits (Holland, 1994; Rasmussen and Buick, 1999).

The formation of Archean and early Proterozoic BIFs places constraints on the sulfur cycle. This is because the development of iron-rich ocean water requires that rates of Fe delivery to the oceans exceeded the rates of sulfide production by sulfate reduction. If rates of sulfide production exceeded rates of Fe delivery, then anoxic ocean waters would become sulfide rather than iron-rich (Canfield, 1998). The great insolubility of iron-sulfide minerals requires that, at normal marine pH values, both iron and sulfide, cannot coexist in abundance in solution; either one or the other will dominate solution chemistry (Canfield and Raiswell, 1991).

Canfield (1998) has recently estimated that the delivery flux of sulfide-reactive iron to the oceans is approx  $5 \times 10^{12}$  mol  $y^{-1}$  of which only about 3 percent comes from hydrothermal sources (table 2). During the Archean and early Proterozoic the delivery flux of iron from hydrothermal sources was probably greater than at present, perhaps by as much as a factor of two (Des Marais, 1997), but a smaller continental area may have provided for a lower flux of terrigenous material. We therefore retain  $5 \times 10^{12}$  mol  $y^{-1}$  as an estimate of the delivery flux of sulfide-reactive iron to the early oceans. This amount of iron can react with  $10 \times 10^{12}$  mol  $y^{-1}$  of sulfide to form pyrite. This rate of sulfide uptake is approximately seven times lower than the rate of sulfate reduction in modern ocean sediments (Canfield, 1998). The great abundance of unpyritized Archean and early Proterozoic BIF demonstrates that iron delivery to the oceans far exceeded rates of sulfide production by sulfate reduction. Therefore,  $10 \times 10^{12}$  mol  $g^{-1}$  is a maximal estimate of the rate of sulfate reduction in Archean and early Proterozoic oceans.

There was a return to BIF deposition in the Neoproterozoic, around 0.75 Ga (Klein and Beukes, 1993). BIF formation was worldwide, with iron-rich sediments depositing during glacial episodes and in particular, during major transgressive events within inter-glacial periods (Klein and Beukes, 1993). As discussed above, the buildup of dissolved iron in an anoxic ocean requires that the delivery flux of iron to the ocean exceeds rates of sulfate reduction. We propose that reduced rates of sulfate reduction during Neoproterozoic BIF formation could have resulted from an overall collapse in surface-water primary production due to extensive ocean ice coverage, as has been recently suggested (Hoffman and others, 1998). Indeed, extensive glaciation of the land

TABLE 2

*Delivery flux of reactive iron to the modern ocean compared to sulfate and in reduction rate (data from Canfield, 1998)*

Reactive Fe from terrigenous sources	$4.9 \times 10^{12}$ mol $yr^{-1}$
Hydrothermal Fe input	$1.7 \times 10^{11}$ mol $yr^{-1}$
Total reactive Fe delivery flux	$5.1 \times 10^{12}$ mol $yr^{-1}$
Maximum sulfide removal as $FeS_2$	$10.2 \times 10^{12}$ mol $yr^{-1}$
Sulfate reduction rate	$73 \times 10^{12}$ mol $yr^{-1}$

The delivery flux of sulfide-reactive Fe to the oceans was computed by combining the average concentration of total Fe in river particulates (4.4 wt%) and the proportion of this total Fe that is sulfide-reactive (0.27) with the flux of terrigenous particulates to ocean. The composition of river particulates is assumed to represent terrigenous material in general. The total flux of terrigenous particulates ( $23 \times 10^{15}$  g  $yr^{-1}$ ) consists of a river-derived component of  $2.0 \times 10^{15}$  g  $yr^{-1}$  and  $1.5 \times 10^{15}$  g  $yr^{-1}$  each from glacial discharge and wind-blown dust. The reactive iron input from mid ocean ridge hydrothermal vents is computed by combining the average concentration of Fe in vent fluids with the fluid flow through the high-temperature vents. The potential maximum removal rate of sulfide (as pyrite,  $FeS_2$ ) is equivalent to twice the delivery flux of Fe to the oceans. Global rates of sulfate reduction are calculated here from a literature compilation of 220 sulfate reduction rate determinations.

surface is also indicated producing what has been termed a Snowball Earth (Kirschvink, 1992; Hoffman and others, 1998). Conceivably, attenuation of the hydrologic cycle limited sulfate input into the oceans and allowed a slow draw-down in sulfate concentration as sulfur was continuously fixed in marine sediments as pyrite. A return to low sulfate concentrations was perhaps a second factor contributing to reduced rates of sulfate reduction.

*Shale pyritization.*—Rates of sulfide production by sulfate reduction in modern continental-margin sediments generally exceed the burial rates of sulfide-reactive iron (Canfield and Raiswell, 1991; fig. 4). We expect, therefore, that the amount of pyrite formed in continental-margin sediments becomes limited by inputs of reactive iron. This is frequently true (Canfield and Raiswell, 1991), but in many instances reactive iron is preserved due to substantial solid-phase sulfide oxidation (Canfield and others, 1993). Except for regions of active fluid-mud re-mobilization such as occurs off the mouth of the Amazon River (Aller, Mackin, and Cox, 1986), sediment mixing by bioturbating animals is probably the most significant process promoting sulfide oxidation and reactive-iron preservation.

In deep-sea sediments, pyrite formation is not limited by reactive iron. This is because sulfate reduction rates are quite low due to the much larger significance of aerobic respiration as a carbon mineralization pathway (Canfield, 1991, 1993). By contrast, rates of sulfate reduction in sulfide-rich euxinic environments are high, exceeding rates of reactive-iron burial. As discussed above, the accumulation of sulfide in anoxic basins requires that rates of sulfate reduction exceed rates of reactive iron delivery.

We conclude that in the absence of bioturbation (fig. 4), promoting substantial sulfide loss, the formation of pyrite in coastal sediments should be iron limited with modern rates of sulfate reduction. Therefore, Archean and early Proterozoic coastal sediments, depositing well before the evolution of bioturbating animals, would have had iron-limited pyrite formation if they had experienced modern-day rates of sulfate reduction.

Raiswell and Canfield (1998) have recently demonstrated that for modern marine sediments (excluding those depositing in euxinic basins), the amount of pyrite that is potentially reactive toward sulfide and can form into pyrite (sulfide-reactive iron) accounts for 6 to 38 percent of the total iron pool (fig. 5). This same amount of iron should form into pyrite under conditions where pyrite-formation becomes iron limited. The most extensive study of Archean and early Proterozoic shale geochemistry comes from the work of Watanabe and others (1997). These shales come from the Transvaal, Ventersdorp, and Witwatersrand Supergroups of the Kaapvaal Craton, South Africa, span an age from 2.2 to 3 Ga, and were deposited mainly in coastal settings. The extent of iron sulfidization (fig. 6) for most of these shales is much lower than expected for iron-limited pyrite formation (compare figs. 5 and 6). Thus, formation of pyrite was only rarely limited by iron, strongly indicating that sulfate reduction rates were generally much lower than modern coastal sediment counterparts. This conclusion is consistent with stable isotope evidence for low sulfate concentrations during the Archean and early Proterozoic and with the low rates of sulfate reduction required of the deposition of BIFs (see above).

Our interpretation of the sulfur chemistry of shales from the Kaapvaal Craton differs from that of Watanabe and others (1997) who have argued that the low sulfur contents of the shales have resulted from substantial pyrite decomposition by acidic solutions at metamorphic temperatures. There is, however, no petrographic evidence for substantial pyrite loss, no independent evidence for acidic leaching of the shales, nor is there any evidence for reprecipitation of remobilized hydrogen sulfide. It is not clear how remobilized sulfide would have been completely removed from this thick sedimentary

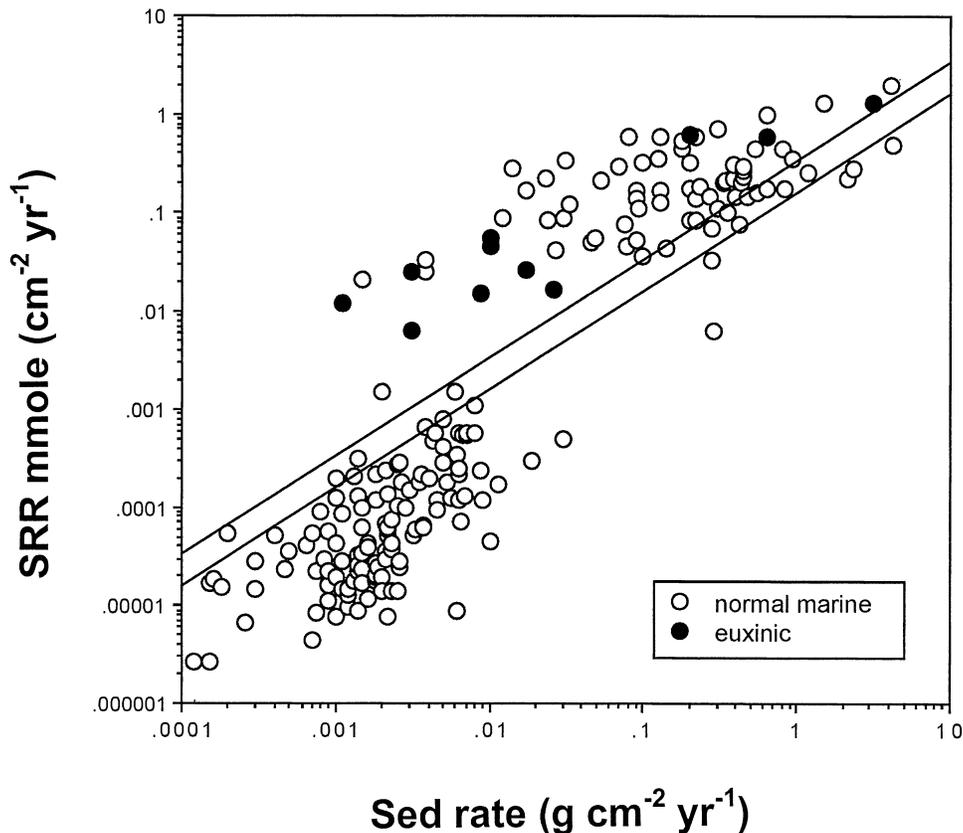


Fig. 4. Rates of sulfate reduction from both normal marine and euxinic settings have been compiled vs. rate of sedimentation. These rates of sulfate reduction are compared with the average reactive iron concentration of  $0.82 \pm 0.37$  wt percent for non-euxinic marine sediments as determined by Raiswell and Canfield (1998). This reactive iron content has been multiplied by a factor of 2 as the mineral pyrite, the most important sulfide phase in marine sediments, has a sulfur to iron stoichiometry of 2 to 1. Sulfide production rates lying above this "iron band" are greater than the ability of the sediment to react with the sulfide to form pyrite. It is obvious that for most modern normal marine sediments depositing at sedimentation rates greater than  $0.01 \text{ g cm}^{-2} \text{ yr}^{-1}$  sulfide formation is potentially iron-limited. All euxinic sediments have sulfide production rates in excess of reactive iron burial rates, and for this reason these environments have sulfide-rich water columns. See text for further details.

sequence without precipitation in the carbonate-rich and Fe oxide-rich lithologies in the section. Furthermore, we are unaware of similar reports of massive sulfide loss from Phanerozoic shales of comparable metamorphic grade (zeolite to middle greenschist facies) and lithology.

Our belief is that a careful study of iron and sulfur systematics in shales of low metamorphic grade depositing throughout the Precambrian will help constrain the intensity of sulfate reduction in ancient environments and will help put further constraints on ocean water-column chemistry (Raiswell and Canfield, 1998). The amount of information on iron and sulfur concentrations in Precambrian shales is presently quite limited, and we suggest that the collection of such data should be a high priority.

*Carbon to sulfur ratios.*—Modern coastal marine sediments deposit with ratios (by weight) of organic carbon to pyrite sulfur (C/S) that vary around a mean of approx 2.8 (Bernier and Raiswell, 1983). By contrast, modern lacustrine sediments have much

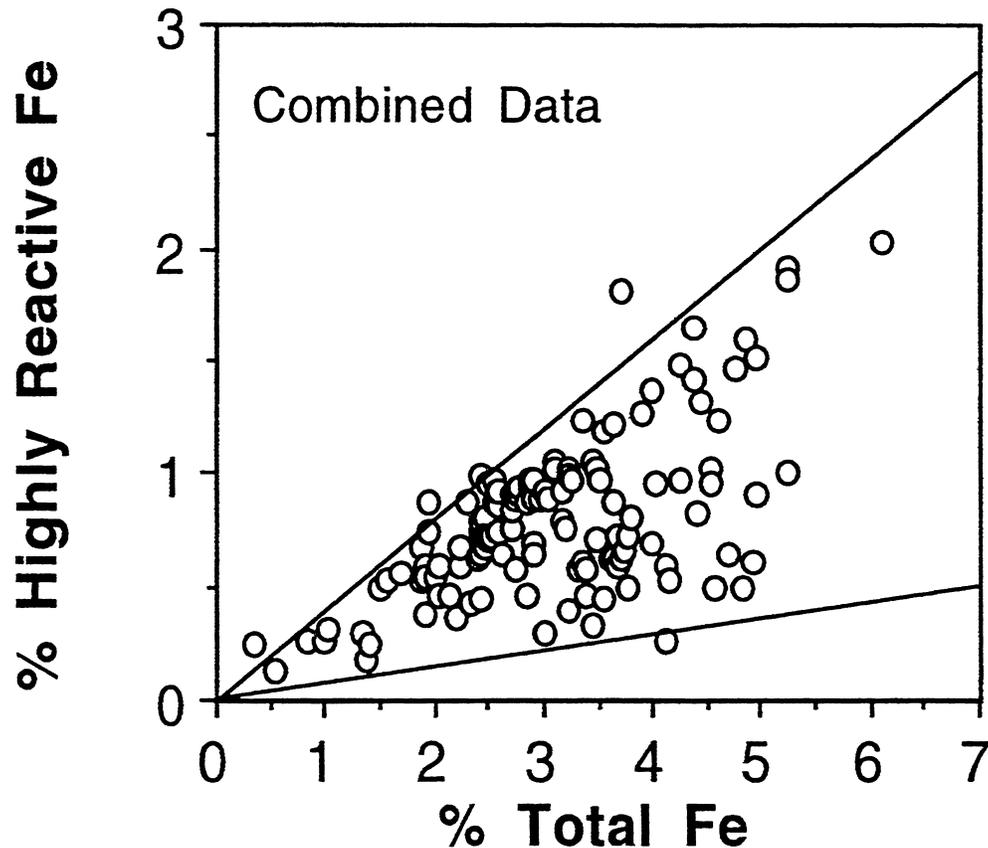


Fig. 5. The highly reactive iron content of over 200 modern marine sediments is shown as a function of the total iron content. Included in the data set are deep-sea and coastal marine sediments underlying well-oxygenated water, and sediments underlined oxygen depleted, but not sulfidic, water columns. Nearly all of the data is bracketed between lines representing, at the low-end, 6 percent of the total iron content, and at the high-end, 38 percent of the total iron content. Highly reactive iron is the fraction of total iron that will react with sulfide on early diagenetic time scales and includes iron that has already reacted to form sulfide plus the remaining iron-oxide fraction extracted with dithionite. This figure is taken from Raiswell and Canfield (1998).

higher C/S ratios, presumably due to lower rates of sulfate reduction promoted by low sulfate concentrations. Owing to this difference, C/S ratios provide a means by which marine and freshwater sediments may be distinguished (Berner and Raiswell, 1983). It has been tempting to use C/S ratios in Precambrian sediments to deduce paleo-sulfate levels and to help constrain ancient rates of sulfate reduction (Strauss and Beukes, 1996; Strauss and others, 1992; Watanabe and others, 1997; Jackson and Raiswell, 1991).

One problem in using C/S ratios in Precambrian sediments to infer the intensity of ancient sulfur cycling is that the C/S ratios of modern sediments, the metric of comparison, are derived in the face of substantial sulfide loss (Morse and Berner, 1995; see above). Thus, 90 percent or greater of the sulfide produced by sulfate reduction in modern sediments is typically lost and not retained as sulfide minerals (Jørgensen, 1982; Berner and Westrich, 1985; Lin and Morse, 1991). Sulfide loss is promoted both by sediment mixing by bioturbating animals and by iron limitation (see above). In one example, Lin and Morse (1991) have documented that between 74 to 99.97 percent of the sulfide produced in sediments from the Gulf of Mexico is lost (table 3). The C/S ratios

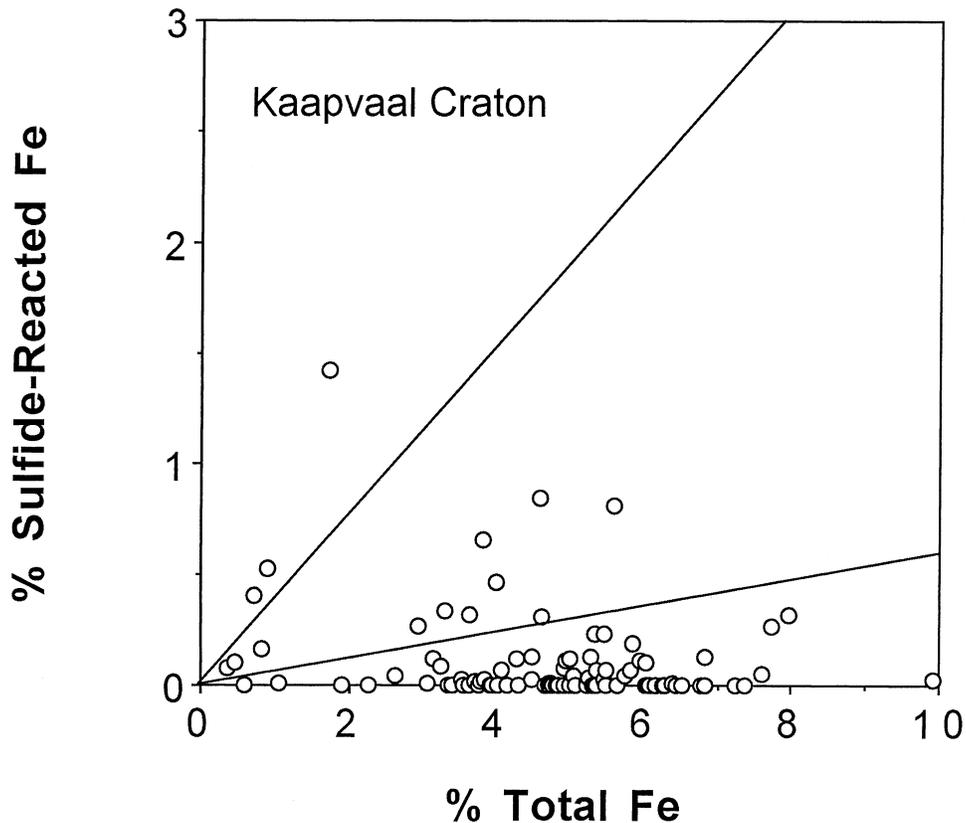


Fig. 6. This figure shows the percent of sulfide-reactive iron versus total iron for a suite of Precambrian shales spanning an age range of 3.0 to 2.2 Ga (Watanabe et al., 1997). The shales are all from the Transvaal, Ventersdorp, and Witwatersrand Supergroups of the Kaapvaal Craton, South Africa. Comparison between this figure and figure 4 shows that, on average, only a small amount of the highly reactive iron fraction has been reacted to form sulfide minerals in these sediments.

in these sediments have also been determined and cluster around the value of 2.8 considered typical of modern normal marine sediments. However, sulfide loss is so great that, in most cases, only 1 percent or less of the measured sulfate reduction rate is necessary to generate the measured C/S ratio (table 3). Therefore, we conclude that the C/S ratios typical of modern marine sediments can be generated with much reduced rates of sulfate reduction provided that sulfide is not lost. In Precambrian sediments free of bioturbating organisms, sulfide retention was necessarily more efficient than in modern sediment counterparts. Therefore, Precambrian sediments with modern C/S ratios do not necessarily represent occurrences of high, near-modern, rates of sulfate reduction.

*Other indicators of Earth-surface change.*—Other indicators of Earth-surface change have a direct bearing on our understanding of the course of biological evolution and, indirectly, on the evolution of the sulfur cycle. Of primary importance is the antiquity of oxygenic photosynthesis. Recall that SSU rRNA tree of life supports a massive radiation of bacterial life in approximate concert with the evolution of Cyanobacteria (fig. 2). Schopf and Packer (1987) have argued that the co-occurrence of cyanobacterial-like microfossils with stromatolitic structures at 3.5 Ga indicates that oxygenic photosynthesis

TABLE 3  
*Sulfur preservation in sediments from the Gulf of Mexico*

Station	% Org C	% S	C/S	SR Rate (mmol m <sup>-2</sup> d <sup>-1</sup> )	% measured rate*	% S Loss
87G1-14	1.0	0.68	1.5	13.8	27	74
87G1-15	1.2	0.17	7.1	7.7	16	84
87G1-16	1.0	1.0	10	6.0	4	96
87G1-17	1.1	0.07	16	7.2	0.5	99.5
87G1-21	1.3	0.16	6.8	0.92	0.9	99.1
86L2-29	0.33	0.06	5.5	2.1	2.7	97.2
TL1-16	0.8	0.17	4.7	6.2	0.2	97.8
T4-17	0.75	0.35	2.1	7.7	0.2	97.8
T4-18	0.4	0.27	1.5	3.7	0.2	97.8
T4-20	0.23	0.16	1.4	2.5	0.04	99.95
T1-6	0.22	0.05	4.4	0.67	0.04	99.96
T1-7	0.33	0.09	3.7	0.57	0.03	99.97

\*The percentage of the measured rate of sulfate reduction required to yield the C/S ratio measured in the absence of sulfide oxidation.

may have evolved by this time. However, the microfossils themselves are not indicative of a specific metabolism, and while the occurrence of stromatolites may suggest phototactic growth, they need not have been formed from oxygenic photosynthesis (Knoll and Bauld, 1989; Knoll and Canfield, 1998). The earliest geochemical evidence for oxygenic photosynthesis is the highly <sup>13</sup>C-depleted organic matter that is common in the sedimentary record at 2.8 to 2.7 Ga (Hayes, 1983, 1984; Des Marais, 1997). This highly <sup>13</sup>C-depleted organic matter is best explained by a two-step fractionation process where an initial fractionation was imparted during carbon fixation by photosynthesis. This organic matter was then subsequently mineralized by methanogenesis producing methane, and the methane was furthermore oxidized by methanotrophic bacteria. Methanogenesis produces very <sup>13</sup>C-depleted methane (up to 70 permil compared to the organic matter) that would be incorporated by methanotrophic bacteria during methane oxidation. Of importance is that methanotrophy requires oxygen which must have been made available by oxygenic photosynthesis. Further evidence for the establishment of oxygenic photosynthesis by 2.7 Ga is given by the appearance of stromatolites in association with highly <sup>13</sup>C-depleted organic carbon, from a lacustrine environment with no obvious source of reduced electron donors (H<sub>2</sub>S or Fe<sup>2+</sup>) to fuel anoxygenic photosynthesis (Buick, 1992).

Through the Precambrian two episodes of enhanced organic carbon burial may be inferred from the stable isotope record of carbon (Des Marais and others, 1992; Karhu and Holland, 1996; Melezhik and Fallick, 1996; Knoll and others, 1986; Kaufman, 1997). The burial of organic carbon into sedimentary deposits leaves behind oxidized species in the Earth-surface environment (Holland, 1973), and, therefore, periods of enhanced carbon burial are periods of net oxidation of the Earth surface. The earliest episode of enhanced organic carbon burial was between 2.0 and 2.3 Ga. The geologic record provides evidence for pervasive oxidative weathering on the continents between 1.9 and 2.2 Ga (Holland, 1994). The obvious synchrony here is probably more than chance and provides strong support for a major episode of Earth-surface oxidation associated with high rates of organic carbon burial (Holland, 1994). As discussed previously, the stable isotope record of sedimentary sulfides indicates that sulfate first accumulated into the global ocean to concentrations > approx 1 mM at around 2.3 Ga (fig. 3). The accumulation of sulfate into the oceans may be taken as further evidence for early Proterozoic Earth-surface oxidation.

Despite an apparently major accumulation of oxygen into the atmosphere during the early Proterozoic, various lines of biological and geological evidence suggest that

oxygen levels did not surpass approx 10 percent of present-day levels until much later in Earth history (Berkner and Marshall, 1965; Cloud, 1972; Knoll, 1992; Canfield and Teske, 1996). Present-day levels of atmospheric oxygen may not have been reached until the Neoproterozoic (0.54–1.0 Ga) in association with a second major burial episode of organic matter (Knoll and others, 1986; Kaufman, 1997; Derry, Kaufman, and Jakobsen, 1992). An increase in oxygen levels at this time would account for: (1) the initiation of large  $^{34}\text{S}$ -depletions ( $>45$  permil) in sedimentary sulfides, indicating the operation of the oxidative sulfur cycle (fig. 3, table 1); (2) the evolution of colorless sulfur bacteria (see above); and (3) the emergence of large multi-cellular animals (Knoll, 1992; Canfield and Teske, 1996; Knoll and Canfield, 1998).

#### COUPLED BIOLOGICAL AND ENVIRONMENTAL EVOLUTION

In what follows we attempt to synthesize the biological and environmental evolution of the sulfur cycle. The geological record begins in the early Archean (3.8–3.9 Ga), and the Earth at that time was bathed in an anoxic atmosphere, with an anoxic ocean containing low concentrations of sulfate. Prokaryotic organisms of the Domains Archaea and Bacteria were probably the only forms of life. If oxygen-producing Cyanobacteria had evolved by this time, then their metabolic activities were transparent and of minor consequence to the state of Earth-surface oxidation. Large continental land masses had probably not yet formed (Taylor and McLennan, 1995; Collerson and Kamber, 1999), and the rock record records vigorous volcanic activity in both sub-aerial and sub-aqueous settings (Cloud, 1988; Lowe, 1994). Associated with volcanic activity was the introduction of inorganic compounds ( $\text{H}_2$ ,  $\text{CO}_2$ ,  $\text{CH}_4$ ,  $\text{SO}_2$ ,  $\text{H}_2\text{S}$ ) driving the metabolism of early chemolithoautotrophic organisms, including organisms of the sulfur cycle. Thermophiles and hyperthermophiles were probably important components of such early bacterial communities. Possible sulfur metabolisms consistent with SSU rRNA phylogenies and the utilization of volcanogenic constituents include anoxygenic photosynthesis, sulfate reduction, and elemental sulfur reduction.

The accumulation of minimally-fractionated evaporitic sulfates in association with magmatically-derived sulfides in the 3.4 Ga Warrawoona Group of Western Australia provides possible evidence for sulfate formation by anoxygenic phototrophic bacteria. There is no unequivocal evidence for production of sulfide by dissimilatory sulfate reduction at this time. However, given the deep branching of hyperthermophilic sulfate-reducing bacteria (fig. 2) from SSU rRNA phylogenies, their presence in the environment by 3.4 Ga might be expected. The probable occurrence of elemental sulfur-reducing bacteria in ancient hydrothermal settings is deduced mainly from the deep branching of these organisms on the SSU rRNA tree of life.

Sulfides depleted in  $^{34}\text{S}$  and of probable biological origin are first encountered at 2.7 Ga, with the first highly  $^{34}\text{S}$ -depleted sulfides, demonstrating fractionations comparable to those known from sulfate-reducing bacteria, by 2.3 Ga (fig. 3). If sulfate reduction was established in the Archean, then the rates were quite low as evidenced by low amounts of sulfide in shales and the persistent deposition of banded iron formations.

The first geochemical and unequivocal sedimentological evidence for oxygenic photosynthesis dates from around 2.8 to 2.7 Ga, and this may be viewed as the latest time for the evolution of oxygenic photosynthesis. The evolution of oxygenic photosynthesis, like the evolution of life itself, was a singular event and not driven by any obvious environmental stimuli except for the opening of an enormous range of new environments that could support photosynthesis. For the first time photosynthesis was limited only by nutrient and light availability (water is nearly ubiquitous) and not by the availability of electron donors (Knoll and Bauld, 1989). In principle, by freeing photosynthesis from the availability of reduced chemical substances, the global production of

organic carbon could be greatly increased. In this way, the evolution of oxygenic photosynthesis becomes a positive feedback on the oxidation of the Earth-surface environment as the ability to produce more carbon should cause more carbon burial, and this should lead to Earth-surface oxidation. Also of possible significance in promoting Earth-surface oxidation would have been the growth and stabilization of continental platforms where carbon burial could occur (Lowe, 1995; Des Marais, 1997).

Most of the bacterial life we know emerged in approximate concert with the evolution of oxygenic photosynthesis (fig. 1). A dramatic evolution in bacterial life might have been made possible by: (1) the appearance of oxygen in sufficient quantities to fuel important metabolic pathways such as, for example, methanotrophy, and (2) an increase in available ecosystem space that global-ranging carbon production and subsequent carbon deposition onto sediments would provide. It is intriguing, although not certain, that the geologic record houses evidence for this dramatic expansion of bacterial life on Earth.

Although the evidence supports the establishment of oxygenic photosynthesis by around 2.7 Ga, the oxidation of the Earth-surface was quite protracted. Sulfate did not accumulate in the oceans to concentrations  $>$  around 1 mM until about 2.3 Ga, roughly contemporaneous with other indicators of Earth-surface oxidation. By 2.3 Ga, rates of sulfate reduction were still lower than modern-day rates as evidenced by the continued deposition of banded iron formations and the preservation of reactive iron in shales of the continental margin. The deposition of BIFs continued until about 1.8 Ga.

Canfield (1998) has suggested that banded iron formations stopped forming when sulfate reduction rates rose to the point of exceeding the delivery flux of iron to the oceans. A rise in sulfate reduction rates would be promoted by an increase in sulfate concentration beyond 1 mM providing a higher flux of sulfate into sediments. At low concentrations, the rates of delivery of sulfate into sediments may be severely limited by diffusion of sulfate across the sediment-water interface. An increase in the oxidized sulfur reservoir is seen as a natural extension of the continued oxidation of the Earth-surface environment. When the production rate of sulfide exceeded the delivery flux of iron, dissolved iron was removed from the oceans by reaction with sulfide. As a consequence, the oceans became sulfidic.

The competing theory is that iron was removed from the oceans by oxidation with oxygen (Cloud, 1972; Holland, 1984). Canfield (1998) has used a simple ocean box model to show that the oxidation of the deep ocean requires levels of atmospheric oxygen approx  $\frac{1}{3}$  to  $\frac{1}{2}$  of present-day levels. Such high levels of oxygen through the middle Proterozoic would be inconsistent with a later Neoproterozoic rise in oxygen levels to greater than approx 10 percent present-day levels (Berkner and Marshall, 1965; Cloud, 1972; Canfield and Teske, 1996). Luckily, we are making progress in using the geochemistry of iron and sulfur to provide evidence for euxinic depositional conditions (Canfield, Lyons, and Raiswell, 1996; Raiswell and Canfield, 1998). A careful evaluation of middle Proterozoic deep-water sediment geochemistry should either support or refute a protracted period of sulfide-rich deep ocean water.

Neoproterozoic BIF formation requires deep-water anoxia, and if the above model is correct, BIF deposition could be a highly visible expression of anoxic deep-water conditions that had existed through nearly all the Proterozoic. The deposition of banded iron formation in the Neoproterozoic required, as was also true for the Archean and early Proterozoic, that rates of production of sulfide by sulfate reduction were lower than rates of iron delivery to the oceans. As discussed above, these special circumstances in the Neoproterozoic may have been promoted by a massive global glaciation.

In the Neoproterozoic the formation of BIF and global deep-water anoxia ceased when rising atmospheric oxygen levels led to the oxidation of deep waters. Rising levels

of atmospheric oxygen resulted from high rates of organic carbon burial. Biological consequences of these increasing levels of atmospheric oxygen include the emergence of large animals and, quite possibly, an evolutionary radiation of non-photosynthetic sulfide-oxidizing bacteria.

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