Archaeon metabolic evolution of microbial mats

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Microbial mats of coexisting bacteria and archaea date back to the early Archaean: many of the major steps in early evolution probably took place within them. The earliest mats may have formed as biofilms of cooperative chemolithotrophs in hyperthermophile settings, with microbial exploitation of diversifying niches. Anoxygenic photosynthesis using bacteriochlorophyll could have allowed mats, including green gliding bacteria, to colonize anaerobic shallow-water mesothermophile habitats. Exploitation of the Calvin–Benson cycle by purple bacteria allowed diversification of microbial mats, with some organisms in more aerobic habitats, while green sulphur bacteria specialized in anaerobic niches. Cyanobacterial evolution led to more complex mats and plankton, allowing widespread colonization of the globe and the creation of further aerobic habitat. Microbial mat structure may reflect this evolutionary development in broad terms, with anaerobic lower levels occupied by archaeal and bacterial respirers, fermenters and green bacteria, while the higher levels contain aerobic purple bacteria and are dominated by cyanobacteria. A possible origin of eukaryotes is from a fusion of symbiotic partners living across a redox boundary in a mat. The geological record of Archaean mats may be present as isotopic fingerprints: with the presence of cyanobacteria, mats may have had a nearly modern structure as early as 3.5 Ga ago (1 Ga = 10^9 years).

Keywords: Archaean; microbial mats; photosynthesis; hyperthermophiles

1. INTRODUCTION

Many of the early stages of microbial evolution may have taken place in biofilms and microbial mats which were highly structured communities of prokaryotes. There are many niche habitats in which consortia of bacteria and archaea may have developed complex specialist metabolic skills. The purpose of this work is to consider the possible ways in which biofilms and mats developed and whether relict traces of the evolutionary history of such communities can be seen, either in the geological record or in the nature and life of extant organisms. It is possible that many important steps in evolution took place within microbial mats, where diverse microorganisms and microhabitats occurred in close proximity.

Microbial mats are of great antiquity. Photosynthetic mats, recorded as stromatolitic carbonates built by cyanobacteria, are abundant in the geological record and some may be as old as 2.9–3.2 Ga (1 Ga = 10^9 years) or more (Awramik 1992; Wilks & Nisbet 1985; Lowe 1994). There is isotopic evidence (Schidlowski & Aharon 1992) to suggest that the operation of autotrophic carbon fixation (and, by implication, oxygenic photosynthesis) on a global scale may date back to 3.5 Ga or earlier. Sulphate-reducing bacteria may be even older. Unfortunately, apart from stromatolites, microbial mats leave little detailed structural evidence. Possible traces have been recorded in Archaean rocks only as carbon–sulphur stringers and sulphide-rich bands with characteristic biogenic isotopic signatures, but these have typically recrystallized and sheared during tectonic events.

2. THE EARLIEST MATS

(a) *Physical and chemical setting*

Hydrothermal vents occur in many settings where hot water exits from circulation systems near cooling magma. Vents are found today around both subaerial volcanoes (where the water inflow is from rain or snow) and submarine volcanism, particularly at mid-ocean ridges. On the early Earth, 4 Ga ago, hydrothermal vents would have been very widespread, around submarine volcanoes, near plume volcanoes (comparable to modern Hawaii) and, possibly, also in subaerial settings over early subduction zones.

It is possible that an early hyperthermophile and non-photosynthetic microbial community existed around hydrothermal vents in waters and muds at 75–115 °C *ca.* 4 Ga ago (Nisbet & Fowler 1996*a,b*). Models of microbial evolution based on ribosomal RNA (rRNA) (Woese 1987; Pace 1997) strongly imply that the last common ancestor was itself hyperthermophile, living in high temperature (> 75 °C) water very probably near a hydrothermal vent (Stetter 1996). This fits well with the geological view (Nisbet 1987) of a Late Hadean–Early Archaean Earth with widespread hydrothermal activity under relatively cool (< 50 °C) oceans. However, Galtier *et al.* (1999) have argued the contrary position, that the very earliest ancestors may have been mesothermophilic. Moreover, the ‘standard’ model in general can be challenged (Doolittle 1999). Nevertheless, the likelihood is that hyperthermophile metabolism is of great antiquity, implying that an early hydrothermally hosted microbial community existed. The earliest diverse microbial communities, when bacteria and archaea had diverged

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Figure 1. Possible metabolic descent of photosynthetic organisms (after Woese 1987; Blankenship 1992; Barnes *et al.* 1996; Pace 1997; Blankenship & Hartman 1998). The stippled region represents the hyperthermophile setting. The figure is based on what Doolittle (1999) termed the 'standard' model. The onset of anoxygenic photosynthesis (ancestral RCII?) allowed escape from the hyperthermophile setting; the symbiosis of RCII (purple bacteria) and RCI (green sulphur bacteria) formed the ancestral cyanobacterium (see the text for an alternative hypothesis); and chloroplast transfer from a cyanobacterium and mitochondrial transfer from a purple bacterium into a Korarchaeota-like organism formed the Eucarya (assuming amitochondrial eukaryotes are descended from earlier mitochondrial ancestors).

as distinct domains (Woese *et al.* 1990) from the last common ancestor, thus may have been hyperthermophile (figure 1).

The management of redox power is a central concern of a microbial mat. By focusing on redox contrast, a mat community can collectively thrive. The earliest mats must have been simple biofilms (figure 2), originally a single-species microcolony, diversifying into opportunistic aggregations of similar cells growing in favourable sites (e.g. near hydrothermal vents). The redox contrast on which they depended would have been present because the hydrothermal fluids would have come from equilibrium with hot mantle-derived magma, while the seawater must have been in exchange with the air, which may have been CO_2 dominated (Kasting 1993). Had the sea not been relatively cool the planet would have dehydrated by complete hydrogen loss from the top of a moist greenhouse atmosphere. Many models of the Earth's early atmosphere do indeed imply partial photolytic loss of hydrogen from H_2O in the upper atmosphere (Yung *et al.* 1989) and the presence of a small but significant amount of O_2 , made as a residual product of hydrogen loss or by atmospheric reactions. The atmosphere would have exchanged directly with the sea, agitated in waves via bubbles and rain-out of volcanic sulphate and nitrate aerosols, introducing oxidation power.

(b) Initial steps in the development of microbial mats

Geologically, a relatively straightforward chain of events can be postulated in the evolution of microbial mats: each step must have offered a small discrete advantage to the organism, in each case building by modifications of an accidental earlier pre-adaptation. A primitive living community based on inorganic redox power would immediately accumulate reduced dead organic matter, opening up a niche for recycling by anaerobic respirers and fermenters. Even at this early stage, recycling of redox power within the mat—a characteristic feature of modern mats—would have

become important. It is thus possible that even the earliest microbial mats were structured physically, with an upper layer of chemolithotrophic organisms underlain by recyclers such as methanogens (figure 2).

The earliest microbial biofilms (Costerton *et al.* 1994) would have been limited to the close proximity of hydrothermal vents. After the split between the domains, perhaps *ca.* 4 Ga ago, motile hyperthermophile bacteria may have lived in cooperation with assorted archaea which recycled organic debris. For example, modern *Aquifex pyrophillus* is a strict chemolithoautotroph depending on molecular hydrogen, thiosulphate and elemental sulphur as electron donors and low concentrations of oxygen and nitrate as electron acceptors. Sulphuric acid is formed as the end-product in growth on sulphur and thiosulphate (Huber *et al.* 1992). Sulphuric acid and oxygen offer opportunities for reducers feeding on the dead bodies of cells: even at this early stage a diverse set of redox habitats could have existed in a hyperthermophile mat. Early hyperthermophile archaea, like modern *Thermoproteus* (Strauss *et al.* 1992), may have already have been accomplished manipulators of sulphur oxidation states.

Modern *Aquifex* possesses catalase, which is related to haem: although this may be a retrofitted later acquisition, it suggests that Mn and Fe oxide-based chemistry evolved in a primitive setting (figure 3) rich in Mn and Fe oxides (characteristic of black 'smoke' from deep hydrothermal vents). If catalase is ancient, this would imply an early need to manage oxidation products: the bacteria may have been able to evolve oxygen from hydrogen peroxide. If the earliest biofilms were in very reducing settings, any peroxide would be easily dissipated in the ambient environment. On the other hand, if the ambient conditions were not strongly reducing, but microaerobic (i.e. a small amount of O_2 supplied by inorganic processes) or if the biofilm were thick enough to delay the emission of noxious species, then catalase would have been useful. The apparent antiquity of hydrogenase, typically an Ni enzyme, suggests an easily available source of Ni, as early

Figure 2. Possible evolution of microbial mats. (a) Earliest Archaean (ca. 4 Ga ago?): hyperthermophile biofilms near hydrothermal systems. (b) Early Archaean (prior to 3.8 Ga ago?): first photosynthesis in organisms close to vents. (c) Early Archaean: anaerobic and microaerobic photosynthesis further from vents and development of purple and green sulphur bacteria. (d) Mid-Late Archaean (ca. 3.5–3.6 Ga ago?): cyanobacterial mats and plankton.

organisms may have been relatively unskilled at the difficult task of metal extraction from the general environment. Early Archaean komatiite volcanism would have been common, producing sills and flows with Ni sulphide layers: Ni enzymes possibly evolved near a komatiite-hosted hydrothermal circulation.

3. LONG-WAVELENGTH ANAEROBIC PHOTOSYNTHESIS

(a) The start of photosynthesis

Nisbet *et al.* (1995) suggested that photosynthesis began as an adaptation of thermotaxis. Infrared heat detection

around deeper water (high temperature) hydrothermal vents would have been advantageous to motile hyperthermophile organisms. An organism possessing infrared thermotaxis which happened to spread into shallow water hydrothermal systems (for example, where a mid-ocean ridge shallowed near a plume comparable to modern Iceland) may have been accidentally able to adapt primitive pigments, formerly used for infrared thermotaxis, into supplementary infrared photosynthesis using bacteriochlorophyll, first with vent light and then with solar photosynthesis.

The first solar photosynthesis may originally have been facultative in a hyperthermophile, used as a supplementary resource when the organism drifted too far from heat. This would allow colonization of mesothermophile habitats in the photic zone. In this model, the *Chloroflexus* reaction centre in a green gliding bacterium is seen as a distant relative of the primitive parent of photosynthesis. Note, however, that the apparent 'low' position of the branch leading to the green gliding bacteria in rRNA-based phylogenies (figure 1) does not necessarily mean the modern organisms use an ancient mode of photosynthesis. Alternative hypotheses are possible: either the reaction centre used by *Chloroflexus*, now the present end of the branch, simply came later than the reaction centres of green sulphur bacteria or the *Chloroflexus* reaction centre was acquired late by retrotransfer from purple bacteria. The archaea did not develop photosynthesis—bacteriorhodopsin is adequate for supplementing but not wholly supporting a cell. Instead, the archaea occupied new mesothermophile respiring niches created by the expanded bacterial biosphere: later, the Eucarya may have adapted ancestral archaeal rhodopsin into sight.

Chloroflexus is resistant to ultraviolet radiation (Pierson 1994), allowing occupation of the deeper parts (> 10 m) of the Archaean photic zone. However, a population in which the only photosynthesizers were *Chloroflexus*-like organisms would not be able to leave the vicinity of the hydrothermal chemical supply (the hydrothermal ghetto) completely, as it would be very dependent on the supply of chemical nutrients from ambient water and hydrothermal water plumes (which can travel tens of kilometres away from vents along modern mid-ocean ridge valleys). Associated with these early photosynthesizers would have been other microbes, both bacteria and archaea, exploiting biological debris by fermentation and respiration (figure 3).

The product of these associations, should all the microbial genera have lived collectively together, would have been a thin bacterial mat community (figure 2) living on the sea floor in warm water in the photic zone, distal from shallow water hydrothermal systems (e.g. around andesite volcanoes or komatiite rifts). On the wider sea floor, away from the influence of water plumes rich in chemicals from hydrothermal activity, biological pickings would mostly have depended on respiration or fermentation, exploiting redox contrast between dead organic matter and wet sediment. Since the supply of organic matter would have depended on the rain of dead

Figure 3. (a) Earliest Archaean: hyperthermophile communities around volcanic vents in both deep and shallow water and possible development of infrared thermotaxis around a deeper water (ca. 400 °C) vent. (b) Early (pre-3.6 Ga ago) photosynthetic community depending on bacteriochlorophyll and CH₄, H₂S, H₂, etc. (c) Mid-Late Archaean (3.6 Ga ago and later) environment with abundant cyanobacteria forming mats on the sea bottom and as blooms in the sea eventually falling to the bottom in aggregations.

material, sea-floor life would have been sparse at any distance from vents. However, planktonic life would have been possible, living by photosynthesis and the contrast between hydrothermal water plumes and ambient water. In particular, photoferrotrophy may have permitted some escape from the immediate hydrothermal vicinity (Pierson 1994). In reduced waters, Fe²⁺ could have allowed early photoferrotrophs to widen the realm of life away from the near vicinity of the hydrothermal vents. Hartman (1984) suggested that, in this early setting, light rather than oxygen could have coupled the Fe cycle to the carbon cycle, with photosynthetic production of ferric iron followed by bacterial re-reduction.

Geologically, it will be difficult to identify any trace of such life in extant rocks. *Chloroflexus* apparently uses the

acetyl CoA or propionyl CoA cycle to fix carbon (Strauss *et al.* 1992). Isotopically, the *Chloroflexus*-only metabolism would leave a $\delta^{13}\text{C}$ fractionation signature of ca. -14‰ (Pierson 1994). Together with this fingerprint, a record of more fractionated material left from coexisting archaeal methanogens would be expected: attempts to distinguish this signature from material formed by green sulphur bacteria using carbon isotopes alone (presumed later, see below) would be ambiguous and unconvincing. Interestingly, Rosing (1999), studying > 3.7 Ga rocks from West Greenland, reported -19‰ fractionation measured in carbon microparticles found in slaty units interbedded in ironstones. The study of Fe isotopes in ancient biogenic rocks, which is just beginning, may be helpful.

(b) *Evolution of the Calvin–Benson cycle*

The reductive pentose phosphate cycle, which is dependent on RuBisCO (ribulose biphosphate carboxylase/oxygenase, here referred to by its colloquial name rubisco), is a feature of aerobic and microaerobic bacteria (with possible minor exceptions). The monopolistic dominance of carbon management by rubisco on a global scale appears to predate 3.5 Ga ago (Schidlowski & Aharon 1992). Rubisco is probably the commonest modern protein as a result of its role in oxygenic photosynthesis, but is also used by non-photosynthetic organisms (e.g. *Beggiatoa*) so its presence is not necessarily indicative of photosynthesis. Rubisco, which is constructed of large and small subunits, each presumably with a considerable evolutionary pre-history before fully assembled, is a very complex, almost anachronistic enzyme. Unlike the superbly efficient catalase, rubisco is apparently 'inefficient' and capable of either carboxylase or oxygenase functions, typically in photosynthesis and photorespiration. Possibly this inefficiency is a consequence of monopoly—perhaps without competition there was no selective pressure to improve its efficiency. If so, attempts to engineer efficiency into rubisco genetically in modern crop plants may have dramatic atmospheric consequences if the 'improved' rubisco were to escape to the rest of the biosphere.

Hyperthermophile bacteria probably do not use the Calvin–Benson cycle. This suggests (though does not prove) that, if the standard rRNA-based model correctly interprets the steps of metabolic evolution, the evolution of rubisco took place at the branching that led to *Chloroflexus*. Purple bacteria do use rubisco: thus, the evolutionary steps which produced the Calvin–Benson cycle occurred before the branching that led to them (figure 1). Rubisco most probably originated in a relatively oxidizing setting given its association with aerobic organisms. The early Archaean global environment was probably microaerobic (and capable of supplying sulphate) rather than reducing, but the ambient oxygen levels were nevertheless probably low. In evolutionary terms, it is difficult to imagine small advantageous steps that could have led to the development of rubisco in a cell. Rubisco's role in photorespiration or as an NH_3 regulator possibly came first. In an early anaerobic or microaerobic photosynthetic system, based on bacteriochlorophyll, a build-up of waste oxidants or ammonia could occur and become toxic to some mat species, while being exploited by other organisms. Indeed, deliberate toxic emissions of oxidants could be favoured by competition between cell types. In such a setting, the capability to deal with oxygen, peroxide, ammonia and hydrogen would be beneficial. In a cell equipped with primitive rubisco which was originally used as an oxygenase for oxygen management, if the $\text{O}_2\text{:CO}_2$ balance shifted the enzyme's carboxylase action could possibly have been exploited and acetyl CoA-based metabolism may have been supplanted. Nitrogenase may have originally evolved for similar reasons lower in a mat. Competition by toxic poison could have become a partnership in a bacterial community, with local oxidizing micro-environments set up and regulated by cells in the mat. In modern, non-sulphur, purple photosynthetic bacteria the control of photosynthesis, carbon dioxide assimilation and nitrogen fixation appears to be

linked in a partnership that may have ultimately formed a global atmospheric regulator (Joshi & Tabita 1996).

(c) *Purple bacteria: reaction centre II*

The development of the ancestral reaction centre II (RCII), possibly from a *Chloroflexus*-like reaction centre coupled to the evolution of the Calvin–Benson cycle in an early green gliding bacterium, could have led to purple bacteria. Initially, these may have been restricted to very localized aerobic micro-environments. There they could have colonized the oxidized 'waste dumps' in biofilms, which would have thickened and diversified the microbial mat community. The *Chloroflexus*-like organisms survived in the lower layers of the mat, whereas the purple bacteria constructed new complexity around and above themselves.

With the evolution of the purple bacteria and the ability to occupy local aerobic settings created within mats, early bacteriochlorophyll-based photosynthetic microbial mats (figure 2) would have become more self-sufficient. Mats comprising purple bacteria, *Chloroflexus*-like species and sulphate reducers, together with other respirers and fermenters and archaeal methanogens, would have been able to spread widely and to take on a role on a planetary scale. The community would only be linked to hydrothermal systems in that these supplied reduced metals to deeper anoxic water generally. Sulphur cycling and separation of the mat into reduced and oxidized sectors would have enabled productivity to increase. The mats would still have been limited by factors such as the supply of oxidized sulphur compounds, but linked with sulphate reducers could have formed simple sulphuretum systems (communities that cycle sulphur between relatively reduced and relatively oxidized states) in shallow water muds and silts, perhaps in tandem with an Fe-cycling 'ferretum'.

(d) *Green sulphur bacteria: reaction centre I*

Though it may be of great antiquity, reaction centre I (RCI) (with Fe–S at its core) may have evolved from a parent that was structurally related to RCII (Rhee *et al.* 1998). Fe sulphides are characteristic deposits of sulphur mats. Green bacteria have a very high sulphide tolerance and can grow in conditions which are toxic to purple bacteria. The development of RCI may have taken place in a highly productive Fe- and S-rich mat in the near vicinity of a shallow-water hydrothermal system. Though green sulphur bacteria are tolerant of high sulphur levels, they are obligate anaerobes and very oxygen sensitive (Pringault *et al.* 1998). Even in a mat growing in water from the Archaean ocean (probably anaerobic) any local oxygen release, for example from peroxide, would have limited the spread of green sulphur bacteria to specific lower layers in the microbial mat. However, in these specific anoxic and perhaps sulphur-rich locations they may have had a considerable advantage. Moreover, by managing the problem of sulphide inhibition of methanogens (McFarland & Jewell 1990) their presence would have favoured the general productivity of the mat (and, thus, amplified their own niche).

By this stage, the Archaean microbial mat (figure 2) would have had a complex set of focused redox levels, with a basal layer of bacterial and archaeal fermenters

and respirers, including methanogens, overlain by green bacteria and *Chloroflexus*-like species and then, in the more aerobic upper layers, sulphate reducers and purple bacteria.

Could such a mat be found in the geological record? Possibly. The $\delta^{13}\text{C}$ isotope fractionation imposed by green sulphur bacteria is relatively small (reductive citric acid cycle: 0 to ca. -15% range) and this signature cannot be easily distinguished in the rock record from the signature of carbon left by clumps of green gliding bacteria. However, coupled with the sulphur isotope fractionation of photosynthesis and parallel sulphate reduction it might be identifiable in rocks that, on sedimentological evidence, are known to have been laid down in shallow water.

5. DEVELOPMENT OF CYANOBACTERIAL MATS

(a) *Evolution of cyanobacteria*

The evolution of oxygenic photosynthesis in cyanobacteria, using chlorophyll rather than bacteriochlorophyll, was crucial in creating the modern biosphere. Cyanobacteria incorporate several distinct components: photosystem I (shared by green bacteria), photosystem II (shared by purple bacteria), the oxygen-evolving centre and the Calvin–Benson cycle. Moreover, heterocysts in many cyanobacteria carry out nitrogen fixation using nitrogenase (a very oxygen-sensitive Fe–Mo enzyme).

One obvious possibility is that this wide array of skills came about as a consequence of the appearance, by mutation, of photosystem I in a cell previously dependent on photosystem II. Green bacteria may be derived from this from a parental two-photosystem cyanobacterial-like cell that then discarded photosystem II. Alternatively (and favoured here, though without supporting evidence) it is possible that green bacteria appeared and then the two-photosystem association came about as a consequence of the symbiotic welding together of a bacterial chimera which included both purple and green bacteria. Such a linkage, if it occurred, could have taken place in a microbial mat micro-consortium living across a redox boundary. Perhaps a green bacterium was an epibiont with a purple bacterium to the extent of fusion.

Blankenship & Hartman (1998) argued that the oxygen-evolving centre originally evolved to oxidize hydrogen peroxide in an organism with a reaction centre similar to that of purple bacteria. Again, a possible hypothesis for the origin of the oxygen-evolving centre is that it may originally have come from toxic warfare between purple bacteria and surrounding anaerobic bacteria. For some species, the production of hydrogen peroxide would be a way of managing oxidation power or of competing with neighbours: in turn, oxygen emission would have been an effective weapon against competing species for purple bacteria. However, cooperation in a micro-consortium living across the fluctuating redox boundary of a mat may also have been possible, with a green epibiont dealing with high-sulphur, very anoxic conditions while the purple host handled more oxidizing microenvironments, the Calvin–Benson cycle of the purple bacteria being initially used in photorespiration to deal with excess oxygen. Once fused, the new cyanobacterium could protect the oxygen-sensitive green epibiont

within and emit oxygen from the oxygen-evolving centre to poison competitors or simply to manage oxidation power.

In what setting did cyanobacteria evolve? Their dominance of mats today is in part because they occupy the upper levels in microbial mats where the energy flux is greater, but, correspondingly, the danger of irradiation is also greater. Could the ancestral role of chlorophyll have been protective for dealing with excess light energy? By effectively incorporating the green bacterium into an organism capable of living in an aerobic habitat while also being able to occupy the uppermost, most energetic, yet most dangerous level in the mat ecosystem, the association would be greatly advantaged.

The various developmental stages outlined broadly parallel the structure of modern microbial mats, with *Chloroflexus* and green and purple bacteria in the lower parts of the mat and oxygenic photosynthesis by cyanobacteria at the top. Possibly, as suggested by Castenholz (1984), this mat structure (figure 2), which is determined by the light-harvesting properties of the bacteriochlorophylls and chlorophylls employed, may in its broadest sense reflect evolutionary history, with progressive upward colonization of the more highly irradiated, more productive upper levels and simultaneous occupation of new niches within the interior of the mat.

The arrival of cyanobacteria and oxygenic photosynthesis would have radically improved the abilities of microbial mats (figure 2) and there was probably explosive colonization of the planet's shallow-water environments by cyanobacteria. The top layers in mats, which are dominated by cyanobacteria, would have created a strongly aerobic local environment for the first time and added oxidation power to the upper layer of ocean water. Below them in the lower levels of photosynthetic mats, the anaerobic parental biosphere continued, its redox state safeguarded by the photosynthetically reduced debris coming from above. With the split into strongly oxidized and reduced parts of the mat, niche exploitation by various recyclers, including sulphur oxidizers and methanotrophs, would be favoured at particular horizons in the mat, in addition to earlier sulphate reducers and methanogens, increasing the total productivity of the mat. Cyanobacteria, being complete consortia within themselves, were pre-adapted to life as plankton (figure 3), exploiting the self-sufficiency of the cyanobacterial cell (Nisbet 1995). Such picoplankton would develop in blooms, eventually sinking in blobs to the sea floor where the photosynthetically reduced carbon would be reoxidized by sulphate reducers.

(b) *Geological evidence of the onset of oxygenic photosynthesis*

The carbon isotopic record in 3.5 Ga rocks (mid-Archaean) is evidence that, by this date, rubisco was acting on a global scale, capturing 20–25% of the flux of carbon atoms coming from the mantle into the atmosphere–ocean system (e.g. Schidlowski & Aharon 1992). The only process that could act on this global scale is oxygenic photosynthesis. Nitrogen-fixing bacteria must also have evolved by 3.5 Ga to support the productivity of the global-scale biosphere as recorded in carbonate carbon isotopes. *Oscillochloris*, which is related to

Chloroflexus, can fix nitrogen (Keppen *et al.* 1994). Cyanobacterial heterocysts are specialist skilled nitrogen fixers, managing to protect nitrogenase in an oxygen-rich setting. Thus, by this stage a near-modern global marine carbon cycle would have been in operation. By this time, complex microbial photosynthetic mats probably existed in shallow water (figure 3). Moreover, diverse cyanobacterial picoplankton may have radiated quickly once the first cyanobacterial cell was formed. All these should have left isotopic traces in the geological record.

6. MATS OF SULPHUR BACTERIA

The antiquity of the sulphur cycle is controversial (Ohmoto & Felder 1987; Ohmoto 1992). Habicht & Canfield (1996) considered sulphide oxidizers to be 'young', of Proterozoic origin. Yet the anaerobic oxidation of sulphur itself may be an old skill, as it is present in both archaea and bacteria such as *Aquifex* (though note that lateral gene transfer is also possible). *Chloroflexus* oxidizes sulphide to elemental sulphur, while *Oscillochloris* can also carry out assimilative sulphate reduction (Keppen *et al.* 1994). With the arrival of oxygenic photosynthesis the biosphere had a much higher productivity. Sulphate and nitrate supply to the uppermost layer of the ocean would have increased, as volcanic aerosols were more efficiently oxidized. If cyanobacterial plankton mats bloomed and sank, the supply of dead reduced organic matter to the sea floor would also have increased. The uppermost layers of the ocean may have become locally more aerobic with the new oxygen supply and, at the boundary between this water and more reduced deeper water, new habitat would have developed.

When sulphide oxidizers comparable to those in modern *Beggiatoa* mats appeared, mats of colourless sulphur bacteria (Gundersen *et al.* 1992; Fenchel & Bernard 1995) would have become widespread, supported by the oxidation power of the global population of cyanobacteria. When the deeper water became oxidized (perhaps not earlier than *ca.* 2.2–2.0 Ga ago, when redbeds first indicate an O₂-rich atmosphere, but possibly much later), microbial mats which included sulphide oxidation would have been possible, not only in photosynthetic depths but also non-photosynthetically in deep water below the light zone (cf. Durand *et al.* 1998). In these non-photosynthetic communities, sulphate reducers would have reacted with the rain of reduced carbon, falling as blobs from plankton blooms, while conversely the sulphur oxidizers returned the sulphur by reacting with reduced sulphur species with oxidation power from the water (sustained by the cyanobacteria). The limitation on productivity would have been the supply of organic debris and of metals and usable nitrogen and phosphate.

7. EVOLUTION OF THE EUKARYA

The Eucarya are of great antiquity, probably dating from before 2.7 Ga ago in the Late Archaean (Brocks *et al.* 1999). Mitochondria and hydrogenosomes appear to have a common ancestor (Bui *et al.* 1996) and may be related to α -proteobacteria such as *Rickettsia*. Recently it has been suggested that, instead of originating through serial endosymbiosis, the formation of the mitochondrion was

simultaneous with the origin of the eukaryote nucleus (Gray *et al.* 1999). In the 'hydrogen hypothesis' (Martin & Muller 1998), anaerobic archaea dependent on hydrogen formed a tight physical association with heterotrophic proteobacteria capable of producing molecular H₂ through anaerobic fermentation. Alternatively, anaerobic archaeobacteria may have evolved the ability to survive in oxidizing environments by incorporating respiring proteobacteria (Vellai & Vida 1999). Though it is unlikely that this evolutionary development was caused by slow oxygen build-up of the atmosphere (*ca.* 2.2 Ga ago, probably after the origin of Eucarya: note that much of the modern biosphere remains anaerobic, with reducing sediment balancing the oxygen-rich air), there is a possibility that both hypotheses may be tenable. If the parent organisms lived in the anaerobic part of a cyanobacterial mat, changing conditions, such as diurnal fluctuations, could have enhanced the productivity of the upper layers. This would have produced a frequent downward shift of the redox boundary, so that underlying anaerobic organisms would have experienced a surplus of oxygen and shortage of hydrogen. In mats with tightly bound communities of diverse prokaryotes, complex local oxygen and hydrogen management partnerships would be likely between cells. It is possible that the eukaryote synthesis came from such a partnership. The new cell, with its hydrogenosome-mitochondrion, would be able to handle a much wider redox range and would be more mobile during diurnal shifts than an aggregation of symbiotic cells. The incorporation of the chloroplast may have followed (or have been simultaneous), from a partnership of the mitochondrion-bearing cell with a cyanobacterium from the upper layers of the mat. With mitochondrion and chloroplast and nitrogen fixing, the eukaryote cell was powerful enough to remake the global atmosphere.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.