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Introduction: how and when did microbes change the world?

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1. BACKGROUND: THE NEED FOR A SYNTHESIS

Living organisms are open systems. They tap the flow of thermonuclear energy from the sun and nuclear fission energy from deep in the Earth's crust to process simple chemicals from their environment and build their cells. In so doing they excrete others, such as carbon dioxide, oxygen, methane, hydrogen, nitrogen, hydrogen sulphide and numerous organic compounds. When did these processes all start? How have they evolved and changed the Earth's surface and climate over the more than 3 Gyr, since life began? Now that agriculture, industry and urbanization are transforming the biosphere and destabilizing the climate, it is more important than ever to understand how living organisms change and are changed by their environment. Until these anthropogenic changes, the effects of macro-organisms were puny compared with the profound impact of micro-organisms: bacteria and protists. For nearly 3 Gyr before animals evolved the world was dominated by microbes of immense metabolic diversity and ultrastructural complexity and variety.

Understanding of the cooperative molecular basis of microbial life has recently deepened through advances in cell biology and genomics. Evolutionary biology and phylogenetic analysis have strikingly improved our ability to reconstruct the family relationships of all organisms and build a realistic picture of the tree of life. Study of fossils, isotopic ratios and chemical biomarkers from petroleum or bitumen provide complementary evidence and real dates for what happened when. Mathematical models for the carbon and other biogeochemical cycles, and their interaction with the Earth's climatic system—plus new empirical data stretching far back in history—are bringing exciting new insights bearing on our own future.

To encourage a synthesis among these diverse but ideally synergistic fields, we organized a conference of a dozen prominent contributors to them on 26–27 September 2005 on the theme of the major steps in cell evolution: when they occurred, and how they transformed the biosphere, the surface of the Earth, and global climate. The present volume contains the papers from this conference.

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One contribution of 14 to a Discussion Meeting Issue 'Major steps in cell evolution'.

2. EARLY LIFE AND THE GREAT OXIDATION EVENT

The most basic question, and one of the most controversial because of the fragmentary and ambiguous nature of the data which potentially speak to it, is when did life begin? At one time, putative evidence for earliest life came from Greenland rocks at Akilia, apparently dating from 3.85 Gyr ago (Mojzsis *et al.* 1996). This is not now generally accepted. Fedo *et al.* (2006) argue that these rocks are not sedimentary banded iron formations, as was first thought (Mojzsis *et al.* 1996), but highly changed igneous rocks that could not have supported life. They suggest that their previous dating was also questionable, because of distortion of isotopic ratios by heat and pressure during metamorphism and replacement of some minerals by younger ones. They consider that the original rock was probably at least 3.6 Gyr old but might actually be younger than other genuinely sedimentary rocks from Greenland, aged about 3.71 Gyr. They also argue that claims for bacterial microfossils prior to 3.5 Gyr are not justified and are based on pseudofossil mineral inclusions. Most claims for carbon enriched in ^{12}C compared with ^{13}C by biological fractionation before 3.5 Gyr ago they consider are also ill-founded: either the samples are from carbonates that may be much younger and intruded into the original rock or the data can plausibly be explained by abiotic processes. Fedo *et al.* (2006) regard only one example of fractionation, in 3.78 ± 0.08 Gyr old turbidites, as a possible indicator for life, but stress that an abiotic origin for all pre-3.5 Gyr ^{13}C depletion cannot yet be ruled out.

A major topic of the meeting was the dramatic rise in oxygen in the Early Proterozoic, 2.45–2.2 Gyr ago: the great oxidation event, reviewed by Holland (2006). He and Kasting & Ono (2006) emphasize that mass-independent fractionation of sulphur isotopes before 2.9 Gyr ago strongly indicates that the early atmosphere was strictly anoxic. Cavalier-Smith (2006) argues that phylogenetic evidence indicates that oxygenic

photosynthesis originated somewhat prior to the divergence of cyanobacteria and uses combined phylogenetic and palaeontological evidence to suggest a possible date of *ca* 2.9 Gyr for the photosystem duplication associated with its origin. Evidence from organic chemical biomarkers reviewed by Summons *et al.* (2006) and isotope proportions in organic carbon discussed in detail by Hayes & Waldbauer (2006) are used by several contributors to argue that cyanobacteria are at least 2.7 Gyr old and against the recent hypothesis that they evolved only *ca* 2.4 Gyr ago, immediately before the great oxidation event (Kopp *et al.* 2005). Fedo *et al.* (2006) argue that claims from lead isotope analyses for atmospheric oxidizing conditions 3.7 Gyr ago are unsound and that a more thorough analysis of the evidence is consistent with the atmosphere then being anoxic. Although one dissenting voice was heard suggesting a much earlier presence of atmospheric oxygen (Ohmoto 2004; Ohmoto *et al.* 2004), there was general consensus that the palaeontological and phylogenetic evidence both strongly support the anoxic nature of the atmosphere and the lack of clear evidence for cyanobacteria before *ca* 2.9 Gyr.

But was life itself present at all before that date? This is a difficult question, because it pushes techniques, data and interpretation to their limits. Schopf (2006) reviews putative cellular fossils and stromatolites, both found almost throughout the period 2.5–3.5 Gyr, albeit much more patchily before 3.1 Gyr. None of the putative cellular fossils in that period can be confidently assigned to any particular bacterial phylum. There appears to be less good preservation prior to about 2.9 Gyr, though Schopf (2006) lists 10 known rock units dated 3.2–3.5 Gyr from which putative microfossils were suggested during the 1980s and 1990s. The third oldest and best studied of these, the Apex chert with its suggested 'filamentous prokaryotes' (Schopf 1992) or 'probable cyanobacteria' (Schopf 1999) was reevaluated by Brasier *et al.* (2002, 2004), who argued that neither their morphology nor their geological context supports biogenicity or cellularity. Brasier *et al.* (2006) consider that a good case has not yet been made for the biogenicity of the other examples prior to 3 Gyr, and that most are indistinguishable from those of the Apex chert. These authors argue that abiotic processes can create morphology of comparable complexity, making biogenicity hard to determine. They also argue that comparable difficulties exist for many stromatolites, and assert that those older than 3 Gyr are less clearly biogenic than later ones. Thus, stromatolites before 3 Gyr might either be a product of largely abiogenic physicochemical processes (Brasier *et al.* 2006) or the organisms putatively involved in their formation may have been physiologically very different from those dominating later stromatolites (Schopf 2006). Schopf (2006) emphasizes that conical stromatolites, in particular, have not been explained by abiogenic processes (which would have to move matter upslope on a macroscopic scale to generate them, in the way that gliding bacteria can easily do) and may be one of the strongest pieces of evidence that life goes back to more than 3.44 Gyr ago.

A new line of enquiry for early life is opened up by Brasier *et al.* (2006), who describe microtubes in 3.4 Gyr quartz sand grains from shallow marine sediments in Western Australia. These microtubes resemble those recently reported by Furnes *et al.* (2004) from *ca* 3.5 Gyr basalts in South Africa but appear better preserved and in a clearer context. Do these provide evidence for endolithic (boring) bacteria as long ago as 3.46 Gyr, as Brasier *et al.* tentatively suggest? Or can abiotic explanations be found? Although contemporary bacteria that bore in concrete or limestone are well studied, little is yet known about those able to bore into silica glass, e.g. volcanic glass substrates (Fisk *et al.* 1998), while modern borings into quartz sand grains are unknown.

3. BIOGEOCHEMICAL CYCLES AND PALAEOCLIMATES

Past models of the history of the carbon cycle have concentrated on crustal, oceanic and atmospheric processes. There is a slow part of the carbon cycle involving weathering of rocks, which is a major sink for atmospheric carbon dioxide, and sediment burial that removes large amounts of organic carbon for long periods. Since oxygenic photosynthesis was invented, sequestering of reduced carbon by burial has allowed oxygen to accumulate in the atmosphere (Holland 2006). The fast part of the carbon cycle is mediated by photosynthesis and other forms of autotrophy that fix carbon dioxide and by respiration and fermentation that return it to the atmosphere. Hayes & Waldbauer (2006) point out that understanding the overall history of the carbon cycle also depends on quantifying the still longer-term processes of supply of new crust rocks from the mantle and their subductive removal into the mantle. They stress that as geothermal processes are continually adding CO_2 from the mantle, redox balance demands that oxidizing power must be being recycled into the mantle or lost to outer space. Hayes & Waldbauer (2006) develop a new quantitative model for the long-term history of the carbon cycle that takes account of carbon additions by volcanic and other geothermal processes and removal by subduction, and apply this to recent data on the rate of these processes. They also emphasize the huge reservoirs of reducing power in the form of ferrous iron and sulphide in the Earth's crust, and argue that these should have caused a very large delay in atmospheric and crustal oxidation after oxygenic photosynthesis evolved.

Hayes & Waldbauer (2006) interpret the negative carbon isotope excursion in inorganic carbon about 2.77 Gyr ago in terms of Hayes' (1983, 1994) original model involving CO_2 fixation by methanogenic archaebacteria, a process strongly biased towards ^{12}C, and the subsequent incorporation into organic molecules of their light methane waste product by methanotrophic bacteria. By contrast, Cavalier-Smith (2006), thinks that archaebacteria are much younger than 2.77 Gyr and thus their activities cannot explain Archaean isotopic history or methane levels. Instead, he puts forward the novel idea that serial depletion by photosynthetic bacteria could explain the spike of extra light inorganic carbon at 2.77 Gyr ago. Hayes & Waldbauer (2006) consider that the broad similarity in fractionation levels 3.5–2.8 Gyr ago to much later

samples is presumptive evidence for Rubisco-based autotrophy, and therefore eubacterial life, during that period.

However, Brasier *et al.* (2006) noted that abiotic causes of all pre-2.8 Gyr ^{13}C depletion in carbonaceous matter are not yet firmly disproved. Cavalier-Smith (2006), while recognizing that evidence for its biogenicity or otherwise is not decisive, argues that interpretations of Early Archaean $^{13}C:^{12}C$ isotope ratios should take into account that the enzyme Rubisco has varied evolutionarily in the degree of ^{13}C depletion it causes by CO_2 fixation, that many of the putatively early chlorobacteria use the less depleting hydroxypropionate pathway, not Rubisco, for CO_2 fixation, and that the expected level of depletion would depend on the proportional contribution of both pathways to organic carbon and on the degree of CO_2 recycling within microbial mats.

There was much discussion about the antiquity of protist microfossils and whether they favour a late origin for eukaryotes around 0.9–1.0 Gyr in the Neoproterozoic (Cavalier-Smith 2006) or a distinctly earlier one in the Mid-Proterozoic (1.8–1.5 Gyr; Knoll *et al.* 2006). The main problem is that the modest diversity of earlier fossils does not strongly resemble any extant eukaryote group, although Knoll *et al.* (2006) argue that they are undeniably eukaryotic. By contrast, Cavalier-Smith (2006) argues that these self-same fossils are at least as likely to be prokaryotes and ties the origin of both eukaryotes and archaebacteria to the Neoproterozoic. Molecular clocks are currently unable to arbitrate decisively between these differing interpretations (Roger & Hug 2006), but are inconsistent with the much earlier 2.7 Gyr old fossil steranes (Summons *et al.* 2006) being eukaryotic.

Gribaldo & Brochier (2006) discuss phylogenetic evidence for the evolutionary diversification of archaebacteria in detail, using phylogenetic trees based simultaneously on numerous evolutionarily conserved genes. Methanogenic bacteria form a single branch of the archaebacterial evolutionary tree, nested within euryarchaeotes, one of the two subphyla of archaebacteria. Although rooting the archaebacterial tree is problematic, like that of the bacterial and eukaryotic trees (Cavalier-Smith 2006; Embley 2006; Roger & Hug 2006), they argue that the first archaebacterium was probably not a methanogen and that methanogens and biogenic methane production post-dated the origin of archaebacteria. Gribaldo & Brochier (2006) point out that anaerobic methanotrophic bacteria, which feed on methane and excrete CO_2, are probably derived from methanogens, and thus must be younger still. Unfortunately, there is no direct evidence for the age of archaebacteria, either from morphological fossils or biomarkers.

Kasting & Ono (2006) discuss the Earth's climate and its regulation in its first 2 Gyr. They explain that, as the sun was then fainter, atmospheric concentrations of CO_2 and/or methane must have been higher for their greenhouse warming effect to have inhibited global freezing. They argue that the origin of oxygenic photosynthesis was ultimately responsible for the 2.4–2.2 Gyr snowball Earth global freezing episodes by removing methane through atmospheric oxidation. Like many, they assume that the source of methane was biogenic. By contrast, Cavalier-Smith (2006) suggests that abiotic sources of methane could have been sufficient in conjunction with the highest levels of CO_2 allowed by the ancient palaeosol data of Holland (2006) to have prevented global freezing until cyanobacteria evolved. He argues that a combination of the reduced iron buffer stressed by Hayes & Waldbauer (2006), and inhibition of the evolution of plankton by UV prior to the ozone layer can explain the lag between the origin of cyanobacteria at 2.71 Gyr suggested by biomarkers (Summons *et al.* 2006) and atmospheric oxygenation, and thus also disputes the hypothesis of a later origin of cyanobacteria 2.4 Gyr ago being a direct trigger for snowball Earth (Kopp *et al.* 2005). Cavalier-Smith (2006) and Kasting & Ono (2006) also discuss the possibility that oxygenic photosynthesis arose slightly earlier and might have caused the world's oldest midlatitude glaciation at 2.9 Gyr ago, offering slightly different mechanisms for how this might have happened; either oxidative methane removal or an indirect effect on the sulphur cycle. Both papers also discuss when biological sulphate oxidation evolved.

4. CELL MORPHOLOGY, BIOMARKERS AND LATER LIFE

Except for a few cyanobacteria, the morphology of prokaryotes is generally too variable or simple to provide a reliable guide to phylum identification from fossils. Therefore, organic chemical biomarkers that are geologically stable and genuinely specific for a particular taxon are potentially very important for reconstructing the past history of bacteria and dating times of origin. Unfortunately, since we still know so little about prokaryotic metabolic diversity, specificity can be difficult to prove. Summons presented new evidence for carotenoid markers that appear to be specific for photosynthetic purple bacteria (members of the proteobacteria) and for green non-sulphur bacteria (members of the sphingobacteria; Brocks *et al.* 2005), which suggests that both groups may date back to 2.7 Gyr; this is consistent with the phylogenetic evidence that they are mutually related and probably both only slightly younger than cyanobacteria (Cavalier-Smith 2006). Summons *et al.* (2006) argue that fossil steranes, dating back to about 2.7 Gyr ago, provide evidence for the prior existence of oxygenic photosynthesis as steroid synthesis requires molecular oxygen. However, they also argue that past claims that cyanobacteria can make sterols are mistaken and stem from cultures often being contaminated by eukaryotes. In accord with this, all three bacterial phyla (divisions) that can make sterols in a limited way (Posibacteria, Planctobacteria, Proteobacteria) diverged after cyanobacteria according to the phylogenetic interpretation of Cavalier-Smith (2006).

However, in the discussion concerning the recent unpublished resampling of *ca* 2.7 Gyr old biomarkers from fresh drill cores, their apparent concentration at the core periphery (Summons) reawakened concerns about their possible mobility, which perhaps questions their syngenicity (Brasier). The causes of this observation need serious investigation, as dating the origin of

oxygenic photosynthesis and of several eubacterial phyla depends on knowing whether these biomarkers are as old as the rocks.

Knoll *et al.* (2006) comprehensively review and reevaluate the acritarch microfossils and macroscopic fossils dating from 1.8 to 0.51 Gyr ago that have traditionally been regarded as putatively eukaryotic. There is a marked increase in the frequency and morphological diversity of such fossils at 800–850 Myr ago. There is broad agreement that the majority of the morphologically complex acritarchs that post-date 850 Myr are probably eukaryotic, even though most cannot be assigned confidently to a specific eukaryote group. But, the interpretation of the 1.8–0.8 Gyr fossils was the subject of lively disagreement at the meeting. Cavalier-Smith (2006) argued that none of them are definitely eukaryotic, that none are crown group eukaryotes, and that all are probably prokaryotic, even though the possibility that some may be stem eukaryotes cannot strictly be excluded; he regards the increased complexity around 850–800 Gyr ago as stemming directly from the origin of eukaryotes shortly before then. He argues that sterol biomarkers dating from *ca* 2.7 Gyr and deemed to be of eukaryotic origin (Summons *et al.* 2006) are actually bacterial. Knoll *et al.* (2006), however, argued that most of the disputed 1.8–0.8 Gyr fossils are probably at least stem eukaryotes and that some may be crown eukaryotes.

The difficulties of such judgments are well illustrated by *Shuiyousphaeridium macroreticulatum*. It has been proposed as being a member of two very different eukaryote crown groups: dinoflagellates (Meng 2005) and probable fungi (Butterfield 2005). However, Knoll *et al.* (2006) stress that these fossils provide little support for any specific attribution, and regard it as either a stem or crown eukaryote. Cavalier-Smith (2006) considers it hard to decide whether it is a stem eukaryote or an unusually complex prokaryote, but thinks the latter possible even though its surface structure appears more complex than in any known extant prokaryote—especially as we do not understand what cell biological properties are necessary for making such structures. To make matters worse its age is particularly poorly constrained. Knoll *et al.* (2006) argue that better stratigraphy should help improve interpretations. They point to an apparent drop in protist complexity during the Neoproterozoic glaciations and a rise of acritarch diversity afterwards. They emphasize that concentric traces attributable to sweeping fronds of attached macroalgae (or similar sessile animals) are not found before the Ediacaran period. This agrees with the inference based on integrating fossil and molecular data (Cavalier-Smith 2006) that chromalveolates probably originated at that time, as did more complex green and red algae—but contradicts the assignment of *Bangiomorpha* to Bangiophyceae (unless it is substantially younger than the current estimates of 1200 Myr) within Rhodophyta, given the similar apparent age of Floridiophyceae and Bangiophyceae on molecular trees. Knoll *et al.* (2006) argue that the broad patterns they describe of increasing protist fossil diversity in the Neoproterozoic are probably real and that, even though records are sparse, such features as the absence of attached macroalgae prior to the Ediacaran period are unlikely to be artefacts of preservation. They consider reports of such algae in much earlier rocks to be misinterpretation.

Roger & Hug (2006) discuss the many problems with gene sequence trees that can lead to distortions, and the much better understanding we now have of their causes. Recent trees using technically superior methods are an improvement on those from earlier days, but much remains to be done. Roger & Hug (2006) stress that inferring dates from molecular trees is even more hazardous and that the quality of published inferences is highly variable, especially through the use of oversimplified evolutionary models. They reanalyse data from some recent studies using a variety of more sophisticated methods to gain insight into the factors that cause such marked differences in estimated dates (and error bounds) among published studies. In some cases, the use of technically better methods leads to better agreement with directly observed fossil dates, but confidence intervals are generally large and there are still some discrepancies that need further study. They doubt whether even the best statistical models currently in use can cope with sharply episodic or quantum evolution of the sort advocated by Cavalier-Smith (2006).

Improved phylogenetic methods and evidence from a vastly larger number of genes, coupled with focused cell biology to investigate organelles, have led phylogeneticists to discard the once favoured hypothesis that early eukaryotes were anaerobes that had not yet evolved mitochondria (Embley 2006). It is now generally accepted that all extant anaerobic eukaryotes are secondarily derived by the conversion of mitochondria into simpler membrane-bounded organelles: hydrogenosomes and mitosomes. Embley (2006) also presents the evidence that all eukaryotes contain a mitochondrial homologue, demonstrating the pivotal role that the mitochondrial endosymbiosis has played in eukaryote evolution, and suggesting that this compartment of endosymbiotic ancestry is essential for contemporary eukaryotes. He suggests that these discoveries mean that we cannot be certain that the host for the mitochondrial endosymbiosis was already a eukaryote. In slight contrast, Cavalier-Smith (2006) argues that the host was probably either a just freshly evolved facultatively aerobic eukaryote, or else a prekaryote cell that was well on the way to becoming one by already having endoplasmic reticulum, peroxisomes and phagotrophy.

It is now agreed that animals evolved from choanozoan protozoa (Cavalier-Smith 2006; Roger & Hug 2006) and that sponges are the most primitive extant animals. Conway Morris (2006) reviews the fossil evidence that virtually all bilaterian animal phyla first arose in the Cambrian. Like Knoll *et al.* (2006), he argues that the absence of animal fossils prior to the Ediacaran is real and not attributable to inadequate preservation and sampling. Conway Morris (2006) reasonably suggests that the frequent discrepancies with estimates assuming molecular clocks are more likely to stem from defects in these assumptions and/or mathematical methods than in the palaeontological data. The increasing rapprochement between estimated dates and the fossil record when the best phylogenetic methods (Roger & Hug 2006), better

data sets, and the most reliable fossil dates are used tends to support this. Conway Morris (2006) leaves it open whether the Ediacaran Vendobiota include any bilateral animals or whether they are exclusively radiate animals or some independent group.

Cavalier-Smith (2006) attempts a grand synthesis of all available data to present a phylogenetic tree for all living organisms consistent with the fossil record. He argues that, given due care and attention to character polarity and phylogenetic analysis, there is generally good agreement with fossils and chemical markers. He concludes that the history of life is divisible into three great ages, punctuated by the origins respectively of glycobacteria and neomura (i.e. archaebacteria plus eukaryotes), which he refers to as the glycobacterial and neomuran revolutions. Both involved major changes in the bacterial cell envelope, and the neomuran revolution also radically changed the proteins that interact with DNA, making it the most dramatic example of quantum evolution (Simpson 1944) in the history of life apart from the origin of the eukaryote cell, which he argues followed fast on its heels. Cavalier-Smith (2006) suggests that the glycobacterial revolution occurred around 2.8 Gyr ago, was immediately followed by the major adaptive radiation of photosynthetic eubacteria, and led after a lag caused by the absence of the ozone layer and the huge crustal stock of reduced iron to the great oxidation event and the Palaeoproterozoic snowball Earth episodes. He proposes that the neomuran revolution and the origins of eukaryotes and archaebacteria were as recent as about 900 Myr ago and that the Neoproterozoic snowball Earth glaciations were caused with very little lag by the origin of biological methanogenesis about 720 Gyr ago, by the mechanisms proposed by Schrag *et al*. (2002). He argues that most protist groups underwent an explosive radiation in the Cambrian at the same time as the classical Cambrian explosion of animals and that the timing of both was set by the origins of the precursor protists such as choanoflagellates and of the first chromalveolates (eukaryotes that arose by the intracellular enslavement of a red alga, e.g. brown seaweeds) that provided novel food very soon after the ending of Neoproterozoic snowball Earth.

5. CONCLUDING REMARKS

It was clear from the lively discussions throughout the meeting that not all participants were agreed on all interpretations put forward by their colleagues and therefore that much further research remains to be done in this exciting multidisciplinary field. We hope, however, that our meeting and the papers now published will stimulate further integration and fruitful cross-fertilization among the many disciplines represented, and thus lead to deeper and more stable insights in the future. Unlike for many previous discussion meetings, we decided not to publish transcripts of the actual discussions in the hope that this would encourage freer discussion during the meeting. In the event, several papers have been significantly revised in the light of the discussions and refereeing process, so some of the discussion points raised could have seemed incongruous when set against the now published papers. Nonetheless, the reader should get some idea of major points of disagreement and/or differing perspectives from this introduction.

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