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7. Ancient Fossil Record and Early Evolution (ca. 3.8 to 0.5 Ga)

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Abstract. Once life appeared, it evolved and diversified. From primitive living entities, an evolutionary path of unknown duration, likely paralleled by the extinction of unsuccessful attempts, led to a last common ancestor that was endowed with the basic properties of all cells. From it, cellular organisms derived in a relative order, chronology and manner that are not yet completely settled. Early life evolution was accompanied by metabolic diversification, i.e. by the development of carbon and energy metabolic pathways that differed from the first, not yet clearly identified, metabolic strategies used. When did the different evolutionary transitions take place? The answer is difficult, since hot controversies have been raised in recent years concerning the reliability of the oldest life traces, regardless of their morphological, isotopic or organic nature, and there are also many competing hypotheses for the evolution of the eukaryotic cell. As a result, there is a need to delimit hypotheses from solid facts and to apply a critical analysis of contrasting data. Hopefully, methodological improvement and the increase of data, including fossil signatures and genomic information, will help reconstructing a better picture of life evolution in early times as well as to, perhaps, date some of the major evolutionary transitions. There are already some certitudes. Modern eukaryotes evolved after bacteria, since their mitochondria derived from ancient bacterial endosymbionts. Once prokaryotes and unicellular eukaryotes had colonized terrestrial ecosystems for millions of years, the first pluricellular animals appeared and radiated, thus inaugurating the Cambrian. The following sections constitute a collection of independent articles providing a general overview of these aspects.

Keywords: Biomarkers, Cambrian explosion, early evolution, microfossil, origin of eukaryotes

7.1. The First Traces of Life

MARK VAN ZUILEN

Approximately a century ago the fossil record, based predominantly on macroscopic morphologic evidence, could only be traced to the beginning of the Cambrian (544 Ma). The record of life therefore comprised only about 12% of the total history of the Earth (4500 Ma). However, the abrupt appearance of complex organisms suggested that more primitive forms of life must have occurred before the Cambrian. Indeed, as more rock formations of Precambrian age were studied, many microfossils and even macrofossils of primitive life forms were found (Schopf, 2000). The understanding of life through time was greatly improved with the development of new types of tracers, or 'biosignatures', which include isotopic, mineralogic and chemical indicators. In addition some indirect geochemical evidence has been used to invoke the presence of life; e.g. the first appearance of oxygenic photosynthesizing bacteria is believed to have preceded the rise of oxygen in the atmosphere at ca. 2.3 Ga ago. Due to these new developments, the start of the evolution of life was slowly pushed back in time (Schopf and Klein, 1992; Knoll, 1999). Beyond the Proterozoic–Archean boundary at 2.5 Ga, however, two fundamental changes do occur that greatly challenge the search for traces of early life.

Firstly, a change in geologic processes due to which a simple interpretation of paleo-environmental conditions is difficult. The Hadean and Archean were dominated by high temperature ultramafic volcanism (e.g. komatiites) and chemical sediment deposition (e.g. Banded Iron Formations – BIF – and cherts). The inferred high geothermal gradient (see Chapter 6.1: evolution in geological mechanisms: the 2.5 Ga transition) and predominantly anoxic surface conditions (see Chapter 6.3: atmospheric and ocean physiochemical evolution) must have shaped the nature and habitat of early forms of life. Hydrothermal settings such as are found today at mid-ocean ridges were common and chemoautotrophic life would have been the most dominant life form on the early Earth (Nisbet and Sleep, 2001). The metabolism of these organisms depends on reduced chemical species (e.g. CH_4, H_2, S, H_2S, Fe^{2+}) that are released during alteration of ocean floor volcanic rocks. The search for traces of early life should therefore be directed to those specific environments (hydrothermal vents, subaerial hotspring deposits, chilled margins of pillow basalts) that were likely to have harbored these primitive groups of organisms.

Secondly, a change in degree of preservation of the rock record due to which conventional paleontological tools become highly ineffective. In the progressively metamorphosed rock record of the Early Archean all currently reported types of biosignatures have been found to be ambiguous. Possible microfossil structures have lost most of their original morphology, organic compounds including molecular biomarkers have turned into kerogen or crystalline graphite (of uncertain origin), and isotope signatures have been blurred by exchange reactions and hydrothermal processes. Furthermore, several abiologic metamorphic reactions have been identified that can produce kerogen or graphite, and specific abiologic processes have been described that can generate complex structures that resemble microfossils. Macroscopic fossil evidence in the form of stromatolites has been controversial as well, since several abiologic processes were identified that resulted in similar degrees of structural complexity. In summary, several processes associated with metamorphism (strain, deformation, hydrothermal fluid circulation, metasomatic mineral deposition, thermal degradation) have made the search for traces of early life a great challenge, and has led to several ongoing controversies.

Currently it is difficult to declare with certainty what the oldest trace of life is, and importantly what its nature and habitat were. Life can be traced unambiguously to approximately 2.7 Ga ago, based on well-described morphological microfossils and especially molecular fossils. Beyond this point many claims for biologic processes have been made, and all of them have to some degree been drawn into question (Fig.7.1). Some of the intriguing but controversial early Archean traces include (1) isotopically light graphite inclusions in older than 3.8 Ga rocks from Akilia island and the Isua Supracrustal Belt in southwest Greenland, (2) kerogenous microstructures, stromatolites and diverse stable isotope ratio anomalies in 3.5 Ga cherts from the Pilbara Granitoid–Greenstone Belt in Western Australia, (3) kerogenous microstructures, stromatolites, and diverse stable isotope ratio anomalies in cherts, as well as microscopic tubes in altered pillow basalts from the 3.4–3.2 Ga Barberton Greenstone Belt in South Africa. The purpose of this chapter is to show the problems associated with the search for the earliest traces of life. Paragraph 7.1.1 describes some commonly used tools (biomarkers, microfossil morphology, isotope ratios), and their limitations with regard to the Archean rock record. Paragraph 7.1.2 is an account of some important field examples of the earliest traces of life. This is by no means a rigorous discussion of all work that has been done on describing the early biosphere on Earth. For a classical account of all fossil evidence of the Archean, the reader is referred to 'The Earth's earliest biosphere' (Schopf, 1983).

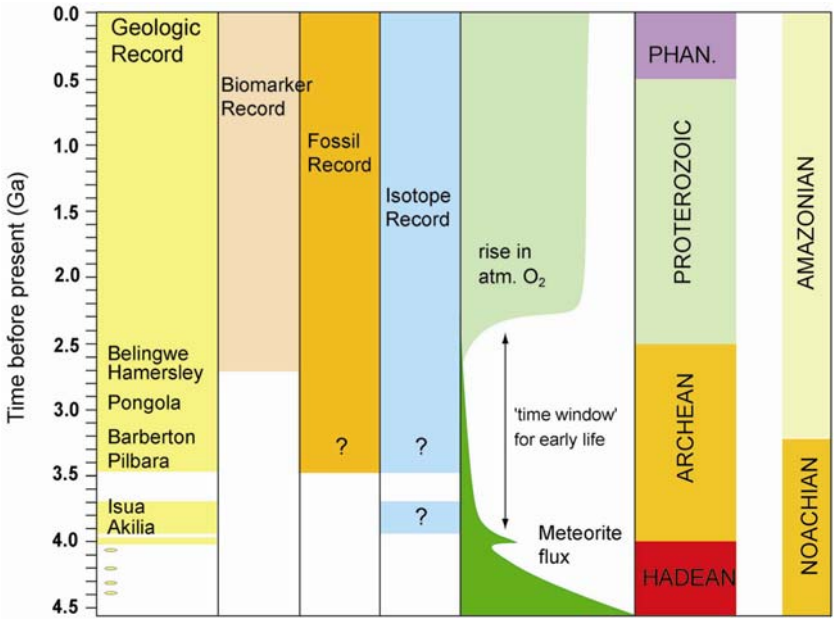

Figure 7.1. A simplified overview of the record of life on Earth over time. Episodes of high meteorite flux to the Earth during the Hadean ceased after ca. 3.9 Ga. Life did not exist or was frequently destroyed during this time (Chyba, 1993). The rise in atmospheric oxygen at ca. 2.3 Ga (Bekker et al., 2004) was caused by oxygenic photosynthesizing bacteria, which are relatively highly evolved organisms. These two events therefore define the 'time window' for the origin and evolution of early life on Earth. Metamorphic alteration of rocks older than 2.7 Ga has caused ambiguity (indicated by question marks) in the interpretation of microfossil and carbon isotopic evidence for life.

7.1.1 The tracers

7.1.1.1 *Morphological fossils*

Macroscopic fossils of Archean age are scarce. Apart from occurrences in the Neoproterozoic of preserved multicellular life (see, for instance Schopf and Klein, 1992; Xiao et al., 1998; Chen et al., 2004), the only macroscopic evidence of Precambrian life comes from stromatolites (Grotzinger and Knoll, 1999). These are laminated, accretionary structures which are commonly regarded to have formed by the sediment-binding or direct carbonate precipitating activities of microbial mats or biofilms composed of photosynthesizing and associated bacteria. Fossil stromatolites only rarely contain individual microfossils. The fine microfabric that is observed in extant stromatolites (Stolz et al., 2001) has been destroyed by diagenesis and metamorphism, leaving only the overall macroscopic appearance of Archean stromatolites as an indicator for biogenicity. The biologic processes that control growth of stromatolites is currently the subject of intense study (see, for instance Reid et al., 2000; Bosak et al., 2004) and different mathematical

models for stromatolite surface growth have been proposed to either argue for (Batchelor et al., 2004) or against (Grotzinger and Rothman, 1996) a biologic control. Several stromatolite structures have been documented in greenstone belts from South Africa and Western Australia that are older than 3.2 Ga. The biologic origin of these structures is the subject of debate, since they only meet several but not all of the criteria for biogenicity (Buick et al., 1981). Abiologic explanations include evaporitic precipitation, soft-sediment deformation, or silicious sinter formation around hot springs (Lowe, 1994).

Most microfossils in silicified Proterozoic microbial mats have relatively simple coccoid or filamentous morphologies and possess a limited number of attributes available for taxonomic characterization. Many characteristics of cell cultures can be modified during post-mortem degradation. Elevated temperature, pressure and strain can cause structures to flatten and ultimately loose their original three-dimensional shape (Schopf and Klein, 1992). These problems have led to many misinterpretations, and to classifications such as 'pseudofossils', 'non-fossils', or 'dubiofossils'. In fact undeformed microfossil shapes in moderately metamorphosed rocks are rather suspicious.

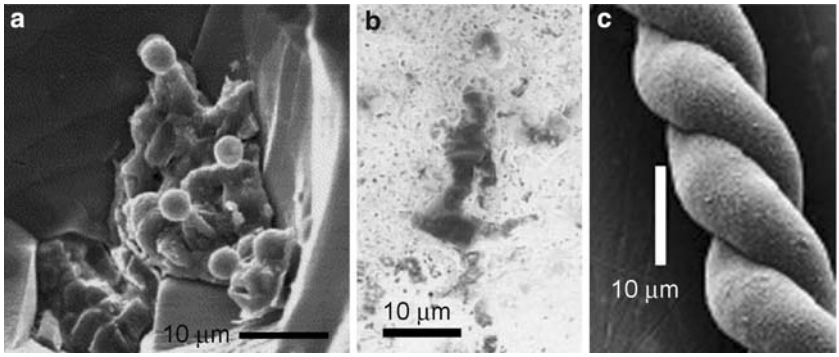

Figure 7.2. Potential problems in microfossil recognition. (a) Endolithic coccoids within a crack in an Isua BIF sample. The coccoids are embedded in extracellular polymeric substances, and although they are partially fossilized they can be recognized as post-metamorphic contamination. (Reprinted from Precambrian Research, Vol. 126, Westall, F. and Folk, R. L., Exogenous carbonaceous microstructures in Early Archaean cherts and BIFs from the Isua Greenstone Belt: implications for the search for life in ancient rocks, pages 313–330, copyright (2003), with permission from Elsevier). (b) Carbonaceous microstructure from the Apex Chert, Pilbara called it the 'ballerina', clearly showing a general problem of morphology; certain 'characteristic' shapes can easily be produced by metamorphic processes. (Reprinted by permission from Macmillan Publishers Ltd: Nature, Brasier M., Green O.R., Jephcoat A. P., Kleppe A., van Kranendonk M. J., Lindsay J. F., Steele A., and Grassineau N. (2002), Questioning the evidence for Earth's oldest fossils, Vol. 416, 76–81, copyright 2002). (c) Microstructures produced by abiologic processes in the laboratory. Such structures can absorb organics, and resemble true Archean microfossils (From Garcia-Ruiz J. M., Hyde S. T., Carnerup A. M., Van Kranendonk M. J., and Welham N. J. (2003) Self: assembled silica-carbonate structures and detection of ancient microfossils. Science 302, 1194–1197).

For instance, spherical objects in a 3.8 Ga metachert from the Isua Supracrustal Belt were interpreted as microfossils (Pflug and Jaeschke-Boyer, 1979). Yet, the rock itself was shown to have experienced intense strain, which should have caused any spherical shape to deform into ellipsoids or more likely rod-shaped objects (Appel et al., 2003). These spheres are therefore clearly epigenetic, and indeed have been interpreted as limonite-stained fluid inclusions, cavities, or post-metamorphic endolithic contamination (Westall and Folk, 2003) (Figure 7.2a). In order to rule out simple shapes such as fluid inclusions or cavities, it has been suggested that putative microfossils should be of organic character. Carbonaceous structures can be recognized using *in-situ* Laser-Raman Spectroscopy (Kudryavtsev et al., 2001; Schopf et al., 2002; Schopf and Kudryavtsev, 2005). However, the Raman spectrum of a putative microfossil can also be derived from abiologic forms of carbon such as graphitic coatings of fluid inclusions (Pasteris and Wopenka, 2002, 2003). Laser-Raman spectroscopy therefore is a necessary but inconclusive analysis for microfossil identification. In addition to simple shapes such as fluid inclusions and cavities, abiologic processes have been recognized that can produce complex microscopic shapes that are capable of absorbing simple abiogenic organic compounds (Figures 7.2b, 7.2c). When metamorphosed such structures display the morphology and the Raman spectrum of a typical microfossil (Brasier et al., 2002; Garcia-Ruiz et al., 2003). In summary, both stromatolites and microfossils are difficult to interpret in the metamorphosed early Archean rock record. For this reason additional chemical, mineralogical, and isotopic tracers have been developed to provide further insight in this part of Earth history.

7.1.1.2 *Molecular fossils*

Molecular fossils, or biomarkers, are derived from characteristic cellular macromolecules, such as membrane lipids. Bacterial and thermal degradation will destroy most biologic material, but some biolipids are transformed into highly resistant geolipids that still carry enough information to identify the original biologic source. Such geolipids have been used extensively to trace life in the geologic rock record. When exposed to higher degrees of thermal alteration, however, these compounds will slowly alter into insoluble macromolecular kerogen (Durand, 1980). As only very small amounts of geolipids can be extracted from metamorphosed material, contamination issues become the biggest hurdle for unambiguous biomarker research of Archean rock samples. Brocks et al. (1999) stressed the importance to identify anthropogenic contamination (e.g. petroleum products from drilling activities), and different forms of post-Archean contamination (including local subsurface biological activity, groundwater containing biolipids, and most importantly migrated petroleum from another source rock that carries geolipids), before a biomarker is recognized as both indigenous to and

syngenetic with the host rock. Currently, small amounts of such unambiguous biomarkers, representative of e.g. cyanobacteria, have been found in the 2.6 Ga Marra Mamba Formation of the Hamersley Group, and the 2.715 Ga Maddina Formation of the Fortesque Group, which both occur in Western Australia (Brocks et al., 1999). Most Archean rocks older than that have experienced more severe metamorphic alteration (lower-greenschist and up), and currently the biomarker record has only been traced to 2.7 Ga (Figure 7.1). New techniques, however, such as hydropyrolytic degradation (Brocks et al., 2003) provide promising venues for obtaining higher yields of extractable geolipids from Archean kerogen. It is therefore certainly possible that the biomarker record will be further extended into deep Archean time.

7.1.1.3 *Isotope ratios*

The carbon isotope ratio $\delta^{13}C$ is expressed as $\delta^{13}C = ([(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{ST}]-1)*1000$ in per mil (‰), relative to a standard (ST = Vienna Pee Dee Belemnite, VPDB). Carbon isotope ratios have been used extensively to trace back life over the geological record (Hayes et al., 1983; Schidlowski, 1988, 2001). The two main reservoirs of carbon in sediments are carbonates with an average $\delta^{13}C$ of 0‰, and the remains of biologic material with an average $\delta^{13}C$ value of −25‰. This characteristic difference in isotope ratio between the two carbon reservoirs has been observed in many organic-rich sediments of different ages and is due to a kinetic isotope effect associated with irreversible enzyme-controlled metabolic pathways of autotrophic organisms (most of them photosynthetic). Carbonaceous material in Archean cherts has a low average $\delta^{13}C$ value of ca. −35 to −30 ‰ (Hayes et al., 1983; Ueno et al., 2004). If it is assumed that mantle-derived CO_2 at that time had a $\delta^{13}C$ similar to that of today (−5 ‰, Des Marais and Morre, 1984), CO_2-fixation by organisms should have produced a carbon isotope fractionation close to −30‰ relative to the source. Photosynthesizing organisms and methanogens are capable of producing this degree of carbon isotope fractionation (House et al., 2003). Unfortunately in moderately to highly metamorphosed rocks these initial isotopic ratios can be lost. Processes that can cause changes include isotope exchange with carbonates or CO_2-rich fluids (Schidlowski et al., 1979; Robert, 1988; Kitchen and Valley, 1995) and devolatilization reactions during metamorphism (Hayes et al., 1983). These processes shift the $\delta^{13}C$ of sedimentary biological material to higher values, making it isotopically indistinguishable from e.g. graphite that forms abiologically during metamorphic processes (van Zuilen et al., 2002, 2003).

The sulfur isotope ratio $\delta^{34}S$ is expressed as $\delta^{34}S = ([(^{34}S/^{32}S)\ \text{sample}/(^{34}S/^{32}S)ST]-1)*1000$ in per mil (‰), relative to a standard (ST = Canyon Diablo troilite, CDT). Sulfate-reducing bacteria preferentially reduce the

light isotope, leading to isotopically depleted sulfides with a range in $\delta^{34}S$ between −10 to −40‰ (Canfield and Raiswell, 1999). In general a small range in $\delta^{34}S$ is observed for igneous rocks of about 0‰ ± 5‰. Therefore it has been suggested by many workers that the significantly low $\delta^{34}S$ values of sedimentary sulfide deposits provide a record of sulfur reducing bacteria over time (Ohmoto et al., 1993; Rasmussen, 2000; Shen et al., 2001). In rocks older than approximately 2.7 Ga most $\delta^{34}S$ values of sedimentary sulfides cluster around mantle sulfur isotope values (0 ‰ with a range of ca. 10‰). Hydrothermal fluid circulation can cause inorganic sulfate reduction and potentially cause isotope effects that fall in the observed range. The search for unambiguous biologic $\delta^{34}S$ signatures is further complicated by metamorphic overprinting. Especially in early Archean deposits it becomes difficult to establish a syngenetic origin of sulfide deposits.

The nitrogen isotope ratio $\delta^{15}N$ is expressed as $\delta^{15}N = ([(^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{ST}]-1)*1000$, in per mil (‰) relative to a standard (ST = nitrogen-air standard, Nier, 1950). Beaumont and Robert (1999) have shown that the $\delta^{15}N$ of kerogen in Archean metasediments is several per mil (‰) lower than that found in the modern biosphere (ca. +5‰), which they suggest is due to the absence of nitrifying and denitrifying bacteria, and the presence of nitrogen fixing bacteria in the mildly reducing Archean oceans. Specific low nitrogen isotope compositions have been observed in certain Archean hydrothermal settings (Pinti et al., 2001). Such isotopic ratios may be indicative of chemo-autotrophic bacteria, which occur in deep sea hydrothermal vent communities and derive NH_3 directly from hydrothermal fluids. However, van Zuilen et al. (2005) have observed low $\delta^{15}N$ in graphites from the 3.8 Ga Isua Supracrustal Belt, and argued that mantle type nitrogen (−5‰) could have been incorporated during secondary metasomatic processes. Nitrogen that is lost from biogenic material by devolatilization during metamorphism, can be incorporated in clay minerals in the form of NH_4^+ were it substitutes for K^+. When these clay minerals recrystallize at high-grade metamorphism this ammonium ion is retained in the resulting mica (e.g. tobelite). It has therefore been suggested that NH_4^+ concentration in micas and the associated $\delta^{15}N$, could act as a potential indirect biomarker in metasedimentary rocks (Papineau et al., 2005 and references therein). However, metamorphism could drive the $\delta^{15}N$ of residual nitrogen in rocks to higher values (Bebout and Fogel, 1992), making it difficult to interpret $\delta^{15}N$ as a biosignature.

The iron isotope ratio $\delta^{56}Fe$ is expressed as $\delta^{56}Fe = ([(^{56}Fe/^{54}Fe)_{sample}/(^{56}Fe/^{54}Fe)_{ST}]-1)*1000$ in per mil (‰) relative to a standard (IRMM-014 reference material). Igneous rocks worldwide have a near-constant $\delta^{56}Fe$ of zero ‰, and geologic processes such as melting/crystallization or weathering do not cause significant isotope fractionation (Johnson et al., 2004b). The isotopic composition of iron is affected by biological processes and can potentially be used to trace life in ancient environments (Johnson et al.,

2004b and references therein). For instance Fe(II)-oxidizing anoxygenic photosynthesizers can cause a shift in δ^{56}Fe of +1.5‰ (Croal et al., 2004). Shifts towards negative δ^{56}Fe are observed for Fe(III)-reducing bacteria (Johnson et al., 2005). A range of δ^{56}Fe values (between ca. −1 and +1‰) has been observed in Precambrian BIFs (Johnson et al., 2003; Dauphas et al., 2004; Yamaguchi et al., 2005); negative values in the more reduced mineral phases (pyrite, siderite) and positive values in oxidized mineral phases (magnetite, hematite). It has been suggested before that BIFs are the direct (Konhauser et al., 2002) or indirect (Beukes, 2004) result of photosynthetic activity. The observed positive shifts in Fe-isotope ratio could be a further confirmation of these hypotheses. However, there are abiologic processes by which BIFs can form. For instance direct photodissociation of ocean surface water by UV-radiation could lead to oxidation and precipitation of BIF (Braterman et al., 1983). It is not known to what extent iron isotopes are fractionationed during such a process, and therefore it remains difficult to use BIFs and their Fe-isotope ratio as a biosignature. Furthermore, there are abiologic redox processes that can cause a shift to positive δ^{56}Fe (Bullen et al., 2001). The analysis of δ^{56}Fe is a relatively new field of research (Anbar, 2004) many aspects of iron isotope fractionation still remain to be studied. The use of δ^{56}Fe therefore remains a promising tool for tracing early Archean life.

7.1.2 Examples from the field

7.1.2.1 *Before 3.8 Ga: Akilia Island, West Greenland*

A highly metamorphosed quartz–pyroxene rock on the southwestern tip of Akilia Island has for long been the center of attention regarding the oldest traces of life on Earth. This five-meter wide outcrop (Figure 7.3) was interpreted as a BIF and was found to contain graphite inclusions within apatite crystals (Mojzsis et al., 1996). The low δ^{13}C of these graphite inclusions suggested a biologic source material that had retained its original carbon isotope signature. This claim has since been the center of controversy, as it was argued that the protolith of this rock was not a BIF, but instead a highly metasomatized ultramafic rock which does not represent a marine depositional setting and would not be able to harbor traces of ancient life (Fedo and Whitehouse, 2002a). Since then, geochemical data has been presented to either argue for or against a sedimentary origin (Fedo and Whitehouse, 2002b; Friend et al., 2002; Mojzsis and Harrison, 2002b; Palin, 2002; Mojzsis et al., 2003; Whitehouse et al., 2005). Recently, iron isotope systematics and trace element ratios have been used to establish more firmly a sedimentary origin. Dauphas et al. (2004) observed δ^{56}Fe up to +1.1‰ in the fine-grained part of this quartz–pyroxene rock, which is in line with values observed in

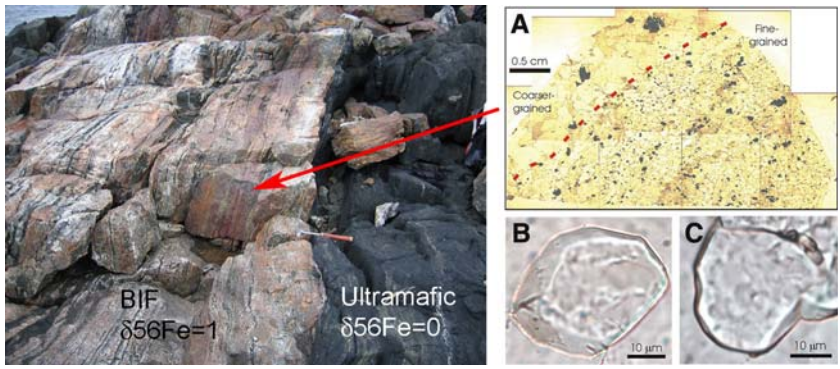

Figure 7.3. (*Left*) Overview of Akilia outcrop, (A) Petrographic thin section of sample G91-26, in which traces of life were found (Lepland et al., 2005). The dashed line shows the contact between fine- and coarser-grained layers. Apatite crystals are common in the fine-grained part, but inclusions of biologically derived graphite are extremely rare. Two of such inclusion-free apatite crystals are shown below (B, C) (Figure 7.3B is reprinted from Lepland A., van Zulien M. A., Arrhenius A., Whitehouse M., and Fedo C. M. (2005) Questioning the evidence for Earth's earliest lif - Akilia revisited. Geology, Vol. 33, 77–79).

younger BIFs (Figure 7.3). It is important to establish that such a positive δ^{56}Fe is not the result of metasomatic alteration of an original igneous protolith. For instance altered mid-ocean ridge basalts (MORB) have positive δ^{56}Fe values (Rouxel et al., 2003). However, such positive δ^{56}Fe correlates with a depletion in Fe concentration. Loss of Fe would be evident in the comparison of the ratios of Fe to an immobile trace element (e.g. Ti, Nb, Hf). If a similar loss of Fe occurred as a result of metasomatic alteration of the quartz–pyroxene rock on Akilia Island, it would be evident in the comparison of the Fe/Ti ratio between this rock and the surrounding igneous rocks. A high Fe/Ti ratio in the quartz–pyroxene rock indicates that Fe was not preferentially lost. On the contrary, the high Fe/Ti ratio resembles those of BIFs found in Isua (Dymek and Klein, 1988).

Apart from the discussion regarding the protolith, the age of this rock is still debated (Whitehouse et al., 1999; Mojzsis and Harrison, 2002a), and the claim for the oldest trace of life on Earth is still strongly contested. As was discussed the positive δ^{56}Fe is in itself not an unambiguous biosignature, since an abiologic process such as photodissociation of ocean water by UV-radiation could have caused iron isotope fractionation. In addition, the graphite inclusions in apatite, claimed to be the remnants of microorganisms, are extremely rare (Lepland et al., 2005; Mojzsis et al., 2005; Nutman and Friend, 2006). Furthermore, it is contested whether the apatite crystals themselves are as old as the surrounding rock matrix (Mojzsis et al., 1999; Sano et al., 1999) casting doubt on their syngenetic character.

7.1.2.2 *3.8 Ga: The Isua Supracrustal Belt, West Greenland*

Early work on the 3.8 Ga old Isua Supracrustal Belt (ISB) in southern West Greenland showed evidence for a marine depositional setting; BIFs, metacherts, pillow lava structures, carbonates, and felsic metasediments in which graded bedding is locally preserved. The occurrences of siderite and dolomite, occasionally interlayered with quartzite in the ISB, appears similar to marine platform deposits that are found throughout the Precambrian and the Phanerozoic, and in early studies this field appearance led to the interpretation of a shallow marine, subtidal depositional environment (Dimroth, 1982). The ISB has a complex metamorphic history; evidence has been reported for multiple episodes of early Archean deformation and metamorphism (Nutman et al., 1996). These events were responsible for amphibolite-facies metamorphism, reaching temperatures between 500°C and 600°C and pressures to 5–5.5 kbar (Boak and Dymek, 1982). Biologic remains in these sedimentary sequences would therefore have been converted to crystalline graphite. It has been suggested in several studies that graphite contained in the ISB could be biogenic in origin (Schidlowski et al., 1979; Hayes et al., 1983; Mojzsis et al., 1996). The wide range of carbon isotope ratios ($\delta^{13}C$ range from -25 to $-6‰$) of graphite in carbonate rich rocks has been interpreted to reflect post-depositional isotopic equilibration of graphitizing organic matter with co-existing carbonates. More recent work has shown inconsistencies in this interpretation (van Zuilen et al., 2002, 2003). Protoliths of several carbonate-rich rocks in Isua have been reinterpreted (Rose et al., 1996; Rosing et al., 1996) as secondary metasomatic and not as sedimentary in origin. This fundamental reinterpretation of the protolith would rule out a biogenic origin of graphite in metasomatic rocks. Graphite was found in large quantities in such metasomatic carbonate-rich rocks, whereas no distinguishable graphite particles were found in sedimentary BIF and metacherts. In these latter samples a very low concentration of reduced carbon was measured (less than 100 ppm), that could be combusted at relatively low temperature (450 °C). Since graphite typically combusts around 700–800 °C and all syngenetic organic material in Isua should have turned into graphite during metamorphic events, it can be concluded that the small amounts of isotopically light reduced carbon in these samples are mainly derived from post-metamorphic (and thus much younger, non-indigenous) organic material. In contrast, the metasomatic carbonate veins within mafic country rocks contain graphite-siderite-magnetite assemblages (Figure 7.4), suggesting that graphite and magnetite in these rocks are the products of partial thermal disproportionation of the carbonate. The siderite ($FeCO_3$) disproportionation reaction, yielding graphite and magnetite ($6FeCO_3 \rightarrow 2Fe_3O_4 + 5CO_2 + C$) has been studied in detail at metamorphic P, T, fO_2-conditions (French, 1971), and has been suggested earlier as a

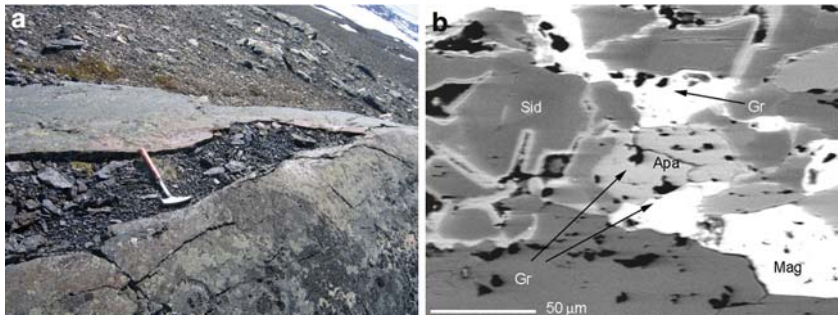

Figure 7.4. a Outcrop of a metacarbonate vein within mafic country rock, eastern part of the ISB. (b) SEM-BSE image of a metacarbonate thinsection. Mineral phases Sid: MgMn–siderite; Apa: apatite; Mag: magnetite; Gr: graphite (Reprinted by permission from Macmillan Publishers Ltd: Nature, van Zulien M. A., Lepland A., and Arrhenius, G., (2002), Reassessing the evidence for the earliest traces of life, vol. 418, pages 627–630).

possible mechanism for graphite formation in the amphibolite facies (T ca. 550 °C; P ca. 5 kBar) ISB (Perry and Ahmad, 1977).

Micrometer-size graphite inclusions with a pronounced light $\delta^{13}C$ value (weighted mean $-30 \pm 3‰$; ion microprobe data) were reported to occur in apatite crystals from the ISB (Mojzsis et al., 1996). The graphite was thought to have escaped isotope exchange with the associated carbonates due to armoring by the host apatite. This claim was based on a rock sample that at the time was believed to represent a sedimentary BIF. More recent petrographic analysis has revealed that it contains MgMn–siderite–magnetite–graphite associations and is compositionally akin to Isua metacarbonates (Lepland et al., 2002; van Zuilen et al., 2002). Furthermore, the REE pattern of these graphite-bearing apatites is distinctly different from apatites occurring in sedimentary rocks (Lepland et al., 2002). As is shown in Figure 7.4b graphite is not restricted to apatite, but occurs as inclusions in most other phases too. The petrographic and geochemical evidence strongly suggests that this graphite is produced epigenetically through thermal disproportionation of ferrous carbonate during one or several thermal events later than 3.8 Ga. The isotopic systematics of the process responsible for formation of isotopically light graphite (weighted mean $-30 \pm 3‰$) enclosed in apatite crystals remains to be studied, but petrographic evidence clearly excludes a primary biogenic origin.

Several other isotopically light graphitic globules have been reported from the Isua region (Rosing, 1999; Ueno et al., 2002). The most intriguing are those that occur in graded beds from the western part of Isua (Rosing, 1999; Rosing and Frei, 2003). This rock outcrop is characterized by significant graphite content, lack of Fe-bearing carbonate, and graphite $\delta^{13}C$ values that are significantly lower than the Fe-carbonate derived graphite described

above. A biologic origin can therefore not be excluded, and further research is necessary to confirm this claim.

7.1.2.3 *3.5 Ga: Pilbara, Western Australia*

Some of the oldest traces of life on Earth have been found in chert horizons (predominantly the Dresser Formation at North Pole Dome, the Apex chert, and the Strelley Pool chert) occurring in the lower Warrawoona Group, eastern Pilbara Craton, Western Australia (Van Kranendonk and Pirajno, 2004), and include stromatolites (Allwood et al., 2006; Walter et al., 1980; Awramik et al., 1983; Lowe, 1983; Hoffman et al., 1999; Van Kranendonk et al., 2003), microfossils of photosynthesizing bacteria (Schopf, 1983, 1993; Schopf et al., 2002), sulfur isotopic evidence of sulfate-reducers (Shen et al., 2001), carbon isotopic evidence for autotrophic life (Hayes et al., 1983; Ueno and Isozaki, 2001; Ueno et al., 2004), and nitrogen isotopic evidence of chemoautotrophic life (Pinti et al., 2001). The validity of these claims strongly depends on the geological context, and especially on the process of formation of the chert horizons in which most of these traces of life are found. The origin of several chert units in the Warrawoona Group has been controversial. The bedded chert–barite horizons in the Dresser Formation were originally interpreted as evaporitic and clastic deposits that were silicified by later hydrothermal circulation (Buick and Dunlop, 1990). In contrast, several lines of evidence suggest that such chert–barite horizons were formed as exhalite deposits associated with hydrothermal seafloor alteration (Van Kranendonk and Pirajno, 2004). This is particularly evident from the swarms of chert–barite veins (Figure 7.5a) that terminate into shallow evaporitic bedded chert–barite units. Such episodes of hydrothermal activity are thought to be associated with caldera formation, as is inferred from chert veins that developed in active growth faults (Nijman et al., 1999). The Apex

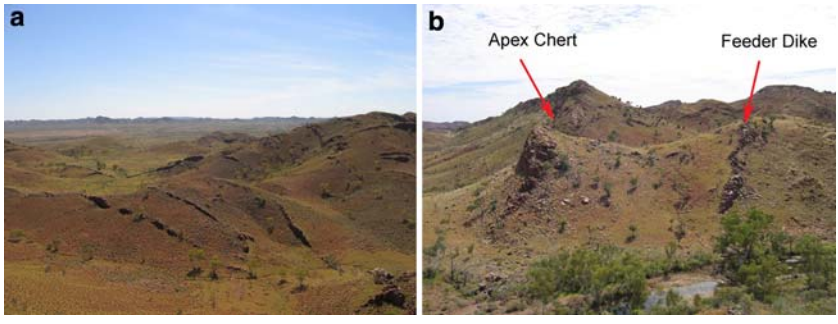

Figure 7.5. (a) Swarms of hydrothermal chert feeder dikes within the Dresser Formation at North Pole Dome, Pilbara, Western Australia. (b) Outcrop of the Apex Chert near Chinaman Creek, Pilbara, Western Australia. Microfossil structures described by Schopf (1993) and Schopf et al. (2002) occur in a hydrothermal feeder dike, not in the actual exhalative seafloor portion of the Apex Chert.

Chert (lower Warrawoona Group) probably formed by a similar process of syngenetic hydrothermal activity, since many feeder dikes have been observed to terminate into it (Figure 7.5b). Recently a similar syngenetic process has been suggested for the origin of black chert dikes and veins within the Strelley Pool chert (Lindsay et al., 2005), although post-depositional hydrothermal activity could have played a role as well. In summary, high temperature ocean floor hydrothermal processes were of key importance for the emplacement of many cherts, and by implication could have been fully or partially responsible for the observed 'biosignatures'.

Most importantly it has been suggested that serpentinization by circulating CO_2-rich fluids at depth in the ocean floor basaltic crust could have prompted hydrocarbon formation by Fischer–Tropsch (FT) type reactions (Figure 7.6a). Modern analogues for this process are mid ocean ridge hydrothermal systems, where abiologic hydrocarbon formation has been observed (Charlou et al., 1998; Holm and Charlou, 2001). FT reactions indeed seem to produce hydrocarbons with a $\delta^{13}C$ range that is similar to biologic material (McCollom and Seewald, 2006). Lindsay et al. (2005) observed low $\delta^{13}C$ carbonaceous clumps and wisps only within a specific depth range of the chert feeder dikes that terminate into the Strelley Pool Chert. They suggest that this depth range corresponds to the optimal conditions for

Abiologic formation of organic compounds:

A) Hydrothermal alteration of ultramafic rocks leads to serpentinization and production of H_2, via the general, non-stoichoimetric reaction scheme (Fo_{88}= 88 Forsterite, 12 Fayalite):

1) Olivine (Fo_{88}) + H_2O = Serpentine + Brucite + Magnetite + H_2

Under these conditions dissolved CO_2 can be reduced to CH_4 and hydrocarbons. This process can be described as a Fischer-Tropsch synthesis, where certain mineral phases (e.g. chromite, magnetite, awaruite) act as catalysts:

2) 12 CO_2 + 37 H_2 = $C_{12}H_{26}$ + 24 H_2O

B) Siderite decomposition in the presence of water vapor (300°C) can generate a variety of organic compounds (McCollom, 2003). A general (non-stoichoimetric) reaction of this kind could take place during partial decomposition of iron carbonates in a hydrothermal system (Garcia-Ruiz et al., 2003) or could lead to graphite formation during moderate to high grade metamorphism (van Zuilen et al., 2002):

3) $FeCO_3$ + H_2O = Fe_3O_4 + CO_2 + CO + H_2 + organic compounds

Figure 7.6. Abiologic formation of organic compounds. (a) FT-type reactions associated with serpentinization produce methane and low molecular weight organics that could have been a source of energy for lithoautotrophic bacteria. Alternatively, high-molecular weight organics and kerogen could have been produced directly by FT-type reactions, providing an entirely abiologic explanation of carbonaceous structures within hydrothermal feeder dikes. (b) Thermal decomposition of iron carbonates in the presence of water vapor can lead to the formation of a complex mixture of hydrocarbons.

FT reactions (*P* ca. 500 kBar, *T* ca. 300 °C). It must be stressed, though, that FT reactions appear to be only efficient in the vapor phase, and are exceedingly more sluggish in hydrothermal systems (McCollom and Seewald, 2001). Ueno et al. (2004) observed low $\delta^{13}C$ carbonaceous structures in the chert feeder dike swarms of the Dresser Formation and noted the absence of an effective catalyst mineral phase and a relatively low hydrothermal temperature (ca. 100–200 °C). They therefore suggest that chemolithoautotrophic organisms may actually have been present in these feeder dikes. The notion that hydrothermal processes could at least in part have produced carbonaceous material in cherts of the Warrawoona Group has led to some important reinterpretations of previously recognized microfossil life. Carbonaceous microstructures resembling fossilized bacteria were reported from the 3.5-Ga-old Apex chert (Schopf, 1993; Schopf et al., 2002). At the time this chert was thought to represent a shallow marine depositional setting in which photosynthetic bacteria could thrive. However, it was subsequently shown by Brasier et al. (2002) that the samples studied by Schopf (1993) actually represent one of the hydrothermal feeder dikes that terminates in a bedded chert horizon (Figure 7.5b). It is highly unlikely that photosynthetic bacteria occurred in such a sub-seafloor hydrothermal setting. Instead, Brasier et al. (2002) argued for the abiologic origin of the observed carbonaceous particles by FT reaction (Figure 7.6). Alternatively, the carbonaceous structures in this feeder dike may have formed by partial decomposition of iron carbonates (as suggested by Garcia-Ruiz et al., 2003, see Figure 7.6b) or may be actually the remnants of chemoautotrophic organisms, in analogy to the suggestion made by Ueno et al. (2004). Many aspects of hydrothermal systems and associated conditions for chemoautotrophic life remain to be studied. As Lindsay et al. (2005) suggest, the abiotic organic output of such systems may overwhelm the signatures of primitive life that are present, and therefore make it the most difficult environments in which to recognize a record of the early biosphere.

Hydrothermal processes would also have caused the emplacement of secondary mineral phases. For instance pyrite with low $\delta^{34}S$ and of putative biological origin could therefore have had a hydrothermal origin. Shen et al. (2001), however, provide compelling evidence that rules out a hydrothermal origin; pyrite grains in bedded chert–barite horizons of the Dresser Formation were found to occur along the original crystal phases of primary gypsum (Buick and Dunlop, 1990). Since gypsum is unstable above 60 °C it implies that these sulfide crystals were emplaced before hydrothermal conversion to barite took place.

7.1.2.4 *3.4–3.2 Ga: Barberton, South Africa*

Traces of life have been found in chert deposits occurring in the Onverwacht Group of the 3.4–3.2 Ga Barberton Greenstone Belt, South Africa (de Wit

and Hart, 1993; Lowe and Byerly, 1999), and include stromatolites (Byerly et al., 1986), microfossils (Walsh, 1992; Westall et al., 2001), sulfur isotopic evidence for sulfate-reducers (Ohmoto et al., 1993), carbon isotopic evidence for autotrophic life (Hayes et al., 1983; Robert, 1988), and stratigraphically constrained carbonaceous structures (Tice et al., 2004; Tice and Lowe, 2004). In addition, (Furnes et al., 2004) described micrometer scale mineralized tubes within the chilled margins of pillow lava structures from basaltic units within the Onverwacht Group.

As was concluded for the traces of life in Pilbara, the validity of these claims strongly depends on the geological context. The Onverwacht Group represents a predominantly thoileiitic and komatiitic volcanic sequence in the lower part of the Swaziland supergroup, that has experienced regional metamorphic alteration as late as 2.7 Ga, when temperatures between 200 °C and 320 °C were reached (Tice et al., 2004). Carbonaceous Chert beds often occur as top layers on volcanic formations (capping cherts) throughout the Onverwacht Group (Figure 7.7a).

Knauth and Lowe (1978) argued that most cherts in the Overwacht Group formed from low-energy diagenetic replacement of preexisting sedimentary and pyroclastic deposits. In the absence of silica-precipitating organisms in the early Archean ocean, silica concentration would be high. Under such circumstances the diffusive flux of silica is directed from ocean water to the sediments (Siever, 1994), leading to silicification of the sediments below (Lowe and Byerly, 1999). In addition to silicified sediments, many underlying volcanic units have been silicified as well. Lowe and Byerly (1986) suggested that these silicified parts of volcanic units represent flow-top alteration zones. Such zones of shallow marine/subaerial alteration formed during intervals of volcanic quiescence. Regional subsidence led to deposition of volcaniclastic material and local growth of stromatolites in marginal evaporitic environ-

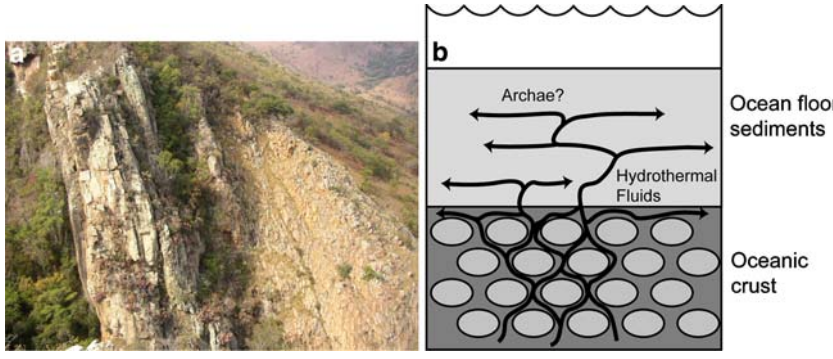

Figure 7.7. (a) Capping chert (left) overlying ocean floor basalt (right) within the Hooggenoeg Formation. (b) Schematic of a convective seawater circulation model (background drawing based on Paris et al., 1985). Hot fluids circulate the basaltic ocean floor and cause serpentinization, and precipitate silica when cooled.

ments. Synsedimentary silicification of these deposits then produced impermeable carbonaceous capping cherts on top of these komatiite flows. In another model Paris et al. (1985) have suggested that silica addition to both volcanic units and overlying sediments is the result of convective seawater circulation that acted directly on the oceanic crust and overlying sediments. Higher heat flux and greater availability of Mg,Fe-rich silicates (ultramafic/mafic basalts) in the early Archean caused effective serpentinization of oceanic crust that produced large quantities of silica-enriched hydrothermal fluids. Lateral migration of such fluids caused silicification of the upper ocean floor basalts and overlying sediments (Figure 7.7b). In addition to this relatively low-temperature hydrothermal circulation model, it has been suggested that some ferruginous chert deposits in Barberton could represent exhalites from local high-temperature hydrothermal vents. This model is based on the occurrence of ironstone pods in close association with BIFs and ferruginous cherts in the region (de Wit et al., 1982; de Ronde and Ebbesen, 1996). The origin of these ironstone pods, however, is still controversial. For instance, it has been suggested that these ironstone bodies are of Quaternary age, and are therefore irrelevant to interpretations of Archean hydrothermal events (Lowe and Byerly, 2003). Alternative explanations for the origin of these iron stone pods, however, have been suggested (Lowe et al., 2003).

As was discussed for Pilbara, serpentinization by circulating CO_2-rich fluids at depth in the ocean floor basaltic crust could have prompted hydrocarbon formation by Fischer–Tropsch (FT) type reactions. However, organics should then be found at a specific depth range within chert feeder dikes that cross-cut the underlying ultramafic/mafic volcanic sequence. Although such carbonaceous veining has been observed, it is clearly not as abundant as in Pilbara. Instead, there is a clear division between the regionally traceable carbonaceous cherts and underlying organics-free volcanic alteration zones. A simple comparison with carbonaceous cherts from Pilbara can therefore not be made, and careful studies are required to resolve the possible biologic origin of the observed organic structures. For example Tice and Lowe (2004) described a stratigraphic sequence within the Buck Reef Chert in the lower Kromberg Formation, and found that carbonaceous debris were restricted to specific shallow marine siderite-dominated successions. The apparent confinement to the photic zone, and the absence of a locally oxidized environment (such as is normally inferred from magnetite or hematite rich BIF's, Beukes, 2004), led them to conclude that anoxygenic photosynthetic life was present at this time. Such organisms use H_2, H_2S or Fe^{2+} as an electron donor for their metabolism. These elements would be readily available in an environment that was dominated by ocean floor hydrothermal alteration processes. The observations by Tice et al. (2004) and by Furnes et al. (2004) suggest that early Archean life may have been intimately linked to ocean floor hydrothermal alteration processes.

7.1.3 The challenges ahead

From the field examples discussed above, it can be concluded that studies of early life in the incomplete and strongly metamorphosed Archean rock record face specific challenges. There is a strong need for careful description of geological context, identification of secondary metamorphic processes, and detailed structural, isotopic and chemical description of microstructures that are indigenous to and syngenetic with the rock formations. One improvement for this field of research is the increased availability of representative, relatively fresh Archean rock samples. Recently several scientific drilling projects have been initiated in Archean terrains, with the specific purpose to study traces of early life. Such drill cores represent samples that have been protected from weathering processes, and are less prone to biologic surface contamination. On the other hand, this type of sampling introduces new problems and challenges. Organic contamination can be avoided by working with well-characterized drilling mud or water. Discrimination between indigenous carbonaceous structures and extant deep biosphere can be achieved by on-site biologic monitoring of drilling mud. Another improvement for this field of research is the wide variety of new isotopic and chemical techniques that can be applied to small rock samples. Laser Raman spectroscopy is now used routinely to determine the organic character (Schopf and Kudryavtsev, 2005) and degree of structure (Beyssac et al., 2002; Tice et al., 2004) of carbonaceous material. It enables the recognition of indigenous metamorphosed structures, and excludes fluid inclusions or post-metamorphic contamination. *In situ* isotopic analysis of putative microfossils is made possible using ion probe techniques. For instance carbon isotope ratios have been determined of individual carbonaceous structures within Archean rock samples (House et al., 2000; Ueno and Isozaki, 2001; Ueno et al., 2002). Detailed sub-micron chemical analysis and chemical mapping of microstructures is achieved using a NanoSIMS (Robert et al., 2005) and detailed sub-micron chemical characterization of fluid inclusions has been achieved for Archean chert samples (Foriel et al., 2004). These *in situ* techniques make it possible to directly link microfossil morphology to both chemistry and isotopic characteristics, greatly improving the discrimination between biologic and abiologic processes. Finally, our understanding of Archean surface processes is greatly expanded with the use of multicollection ICP-MS. This technique enables the precise determination of the natural isotopic variation of e.g. transition metals and other biologically significant elements (Johnson et al., 2004a).

7.2. Microbial and Metabolic Diversification

DANIELL PRIEUR

Organisms living today on Earth use chemicals and/or light to gain the energy required for cellular functions, and they carry out biosynthetic processes converting carbon dioxide or already existing organic compounds. Considered as a whole, the prokaryotes (microscopic unicellular organisms whose genetic material is not separated from cytoplasm by a membrane) possess all types of metabolic pathways known so far. Their study might serve as models for establishing a scenario.

7.2.1 How do contemporary cells gain their energy?

The variety of metabolisms used by prokaryotes has been presented in details by Madigan et al. (2003) in a text book which inspired this paragraph.

7.2.1.1 *Chemotrophic metabolisms*

Chemotrophic organisms gain their energy from oxidation–reduction chemical reactions which obligatorily involve inorganic and/or organic electron donors and acceptors. When molecular oxygen (O_2) is present, it can play as an electron acceptor; then processes are called aerobic respirations. When molecular oxygen is lacking, other molecules (organic or inorganic) play as electron acceptors for processes called anaerobic respirations. In both cases, electrons are transported by electron carriers whose number and type depend on the donor and acceptor involved. During this process, a proton motive force is established across the cytoplasmic membrane, allowing ATP synthesis through an oxidative phosphorylation. When molecular oxygen and other electron acceptors are missing, certain organic molecules can be degraded through an energy generating process called fermentation, in which electron acceptors are provided by intermediate organic compounds deriving from the degradation of the initial organic compound.

7.2.1.2 *Phototrophic metabolisms*

Photosynthesis can be defined as a conversion of light energy into chemical energy: it is one of the most important biological processes on Earth. Organisms carrying out photosynthesis are called phototrophs. Most of them are usually autotrophs, and utilize carbon dioxide as carbon source. Photosynthesis depends on light sensitive pigments present in all phototrophs.

Photosynthesis is the sum of two series of reactions: light reactions and dark reactions. During light reactions, light energy is converted into chemical

energy. When excited by light, some pigment molecules are converted into a strong electron donor with a very electronegative reduction potential. Electrons are then released, and transported by different ways, according to the organisms concerned. This electron flow leads to ATP synthesis.

During dark reactions, chemical energy is utilized to reduce carbon dioxide into organic compounds. Electrons required for carbon dioxide reduction come from the reduced form NADPH (for nicotinamide-adenine dinucleotide-phosphate) of the coenzyme $NADP^+$ (for nicotinamide-adenine dinucleotide-phosphate), previously reduced by electrons whose origin may vary according to the type of photosynthesis concerned.

They are two types of photosynthetic processes. Terrestrial and aquatic green plants, algae, and some prokaryotes of the Bacteria domain (Cyanobacteria) carry out oxygenic photosynthesis. In this case, electrons required for reduction of NADP+ into NADPH are generated by photolysis of water, with the reaction:

$$H_2O \rightarrow 2H^+ + 2e^- + 1/2O_2.$$

Molecular oxygen released during this reaction gives its name to this particular photosynthesis.

The second type of photosynthesis is carried out exclusively by other prokaryotes from the Bacteria domain, called the green bacteria and the purple bacteria. These prokaryotes harbor photo-sensitive pigments (bacteriochlorophylls) that slightly differ from the pigments harbored by oxygenic phototrophs. Transport of electrons released by the light-excited pigments, and following phosphorylation is also different from those encountered during oxygenic photosynthesis. But, in the case of autotrophic growth, purple bacteria use electrons given by reduced molecules present in the environment such as hydrogen sulfide (H_2S) or various organic molecules for reduction of NADP+ into NADPH. This photosynthesis that does not produce molecular oxygen is called anoxygenic photosynthesis.

7.2.1.3 *Electron carriers and ATP synthesis*

During an oxidation–reduction reaction within a cell, electrons are transferred from the donor to the acceptor, by one or more intermediates located in the cytoplasmic membrane and called electron carriers. These carriers are oriented within the membrane in such a way that electrons are transported along this chain, while protons are extruded outside the cell. The result is the formation of a proton gradient and an electrochemical potential across the membrane, with the inside of the cytoplasm alkaline and electrically negative, and the outside of the membrane acidic and electrically positive. This energized state of the membrane is called the proton motive force. This proton motive force is then used to synthesize ATP (adenosine

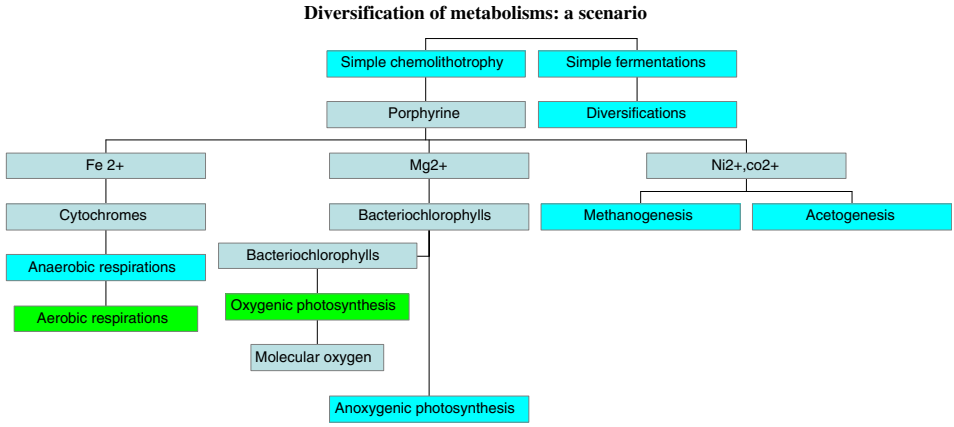

Figure 7.8. Diversification of metabolisms, a tentative scenario.

tri-phosphate), a molecule with high energy phosphate bonds, which represents the most frequent form of energy conservation in the cell. The enzyme that catalyses this reaction is an ATP synthase, or ATPase, which functions as a proton channel from outside to inside the cell, and synthesizes ATP during a process called oxidative phosphorylation.

7.2.2 What were the most probable milestones?

This section is inspired by the hypothesis proposed by Madigan et al. (2003), and summarized in Figure 7.8.

Many actual prokaryotes (both Bacteria and Archaea) utilize molecular hydrogen (and many other inorganic and organic electron donors) as an electron donor, in combination with a variety of electron acceptors such as nitrate, sulfate, ferric iron, and molecular oxygen. Electrons originated from molecular hydrogen are transported towards the final electron acceptor using a variety of specific electron carriers, which number along a particular electron transport chain is rather depending on the difference of reduction potentials between the electron donor and the electron acceptor. Among these electron carriers are the cytochromes. Cytochromes are proteins with iron-containing porphyrin rings that carry out oxidation and reduction through loss or gain of one electron by the iron atom in the porphyrin ring.

Interestingly, other complex molecules involved in energy generating mechanisms are also porphyrin-based. They are particularly chlorophylls and bacteriochlorophylls involved in photosynthesis, and coenzyme F430 involved in methanogenesis, but instead of iron, they include magnesium and nickel, respectively.

Thus the formation of porphyrin-like molecules might have constituted a first step preceding the diversification of anaerobic respirations (including methanogenesis), which allowed living organisms to take advantage of the almost infinite combination of electron donors and acceptors (both inorganic and inorganic) existing on Earth, particularly those yielding large amounts of energy such as molecular hydrogen (donor) and ferric iron (acceptor). Replacement of iron by magnesium within the porphyrin might have led to the formation of photosensitive molecules: the bacteriochlorophylls and the chlorophylls. For the first time, organisms had the possibility to utilize solar light as an unlimited primary energy source.

Photosynthesis (anoxygenic or oxygenic) allows the transformation of photon energy into chemical energy. Briefly, photons excite specific sensitive compounds whose reduction potential becomes electronegative with the excitation, which generates an electron flow, and finally ATP synthesis. Then these phototrophic organisms have to use their energy for synthesizing macromolecules. Some of them can utilize already formed organic compounds from their environment, but many are autotrophs and utilize carbon dioxide as a carbon source, via various biochemical pathways.

Transformation of carbon dioxide into organic carbons requires not only energy, but also a reducing power. In case of molecular hydrogen-oxidizing organisms, hydrogen can reduce carbon dioxide via the electron carriers NADH or NADPH. For some anoxygenic photosynthetic Bacteria (Green sulfur Bacteria and Heliobacteria) the primary electron acceptor is sufficiently electronegative for carbon dioxide reduction through the reverse citric acid cycle or the hydroxypropionate cycle. When an electron donor with a less favorable reduction potential (sulfide, ammonium, etc) is involved, organisms concerned must have recourse to an inverse electron flow. This is the case for purple bacteria (anoxygenic phototrophs), which uptake their reducing power from the environments, using electron donors such as hydrogen sulfide, ferrous iron or organic compounds.

A second, but major step, in the evolution of energy generating metabolisms, was the evolution of oxygenic photosynthesis, carried out by ancestors of the Cyanobacteria. These organisms achieved a mechanism through which the reducing power required for carbon dioxide fixation came from photolysis of water, producing electrons, protons and molecular oxygen as a by-product. This production of molecular oxygen, probably hidden at its beginning by the presence of large amounts of reducing substances and/or its immediate consumption by evolving aerobic organisms, would provide the living organisms the best electron acceptor, in terms of energy generation, and lead to the explosion of aerobic respiration.

Finally, the transfer of energy generating mechanisms invented by prokaryotes (oxygenic photosynthesis and aerobic respiration) to primitive eukaryotes through endosymbiosis, gave the best energetic processes to

lineages that evolved successfully into multicellular complex organisms. One must note that even if today photosynthetic driven life seems to dominate on earth, chemotrophy (organo-but also lithotrophy) is widespread (Karl et al., 1980; Cavanaugh, 1983; Parkes et al., 2000) and are the driving forces of many ecosystems such as deep-sea hydrothermal vents or deep marine sediments (Karl et al., 1980; Cavanaugh, 1983; Parkes et al., 2000).

This tentative scenario might appear feasible but would require supports that will be impossible to obtain. Particularly, one cannot say if the scenario started before or after LUCA (see part 5.7), and how it could have been coupled with the early genome evolution.

The most evident proof of this tentative succession of metabolisms is the increase of molecular oxygen in the atmosphere, following the oxygenic photosynthesis carried out by Cyanobacteria or their ancestors. But one can remind that today, when an aquarium is well balanced (chemically and biologically speaking), nitrite (which results from aerobic ammonium oxidation) is immediately oxidized into nitrate by specific micro-organisms, and is almost or totally undetectable in the aquarium environment. Similarly, the first production of molecular oxygen could have been masked by immediate chemical oxidations, then by the first aerobic respirations, before oxygen production overcame oxygen utilization, and finally reached the equilibrium of the actual atmosphere. For these reasons, the discussion about the first record of Cyanobacteria (or oxygenic photosynthetic organisms) and the oxygenation of the atmosphere is not finished yet.

Also, one must consider the size of micro-organisms and their habitats. A one micrometer microbe is actually depending on physico-chemical conditions which exist in the surrounding micrometers: 1 mm for a micro-organism is equivalent to 1 km for a man. A remote analysis of Earth, and particularly its atmosphere, could not detect the anaerobic life which most probably occurred first and still exist.

7.3. The Origin of Eukaryotes

Purificación López garcía, David Moreira, Patrick Forterre and Emmanuel J. P. Douzery

The terms ‘prokaryote’ and ‘eukaryote’ were introduced with their modern meaning by the microbiologists R. Stanier and C.B. van Niel in 1962 (Stanier and Van Niel, 1962; Sapp, 2005). They reflect the two major structural patterns in cellular organization. Eukaryotes, either unicellular (e.g. microalgae, dinoflagellates, amoebas) or multicellular (e.g. plants, animals, fungi), are characterized by three major features that are missing in prokaryotes: a well-developed cytoskeleton of actin filaments and tubulin microtubules, membrane-bounded organelles (mitochondria, where respiration takes place

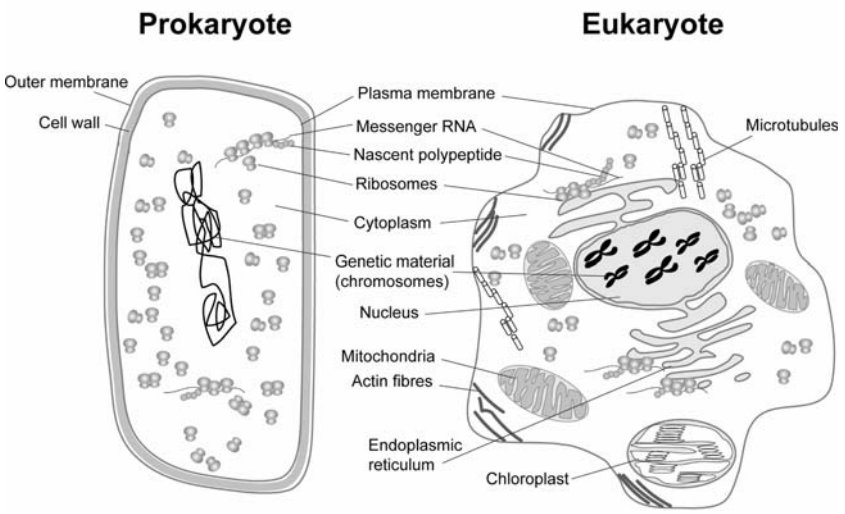

Figure 7.9. Schematic organisation of prokaryotic and eukaryotic cells.

and, in photosynthetic eukaryotes, chloroplasts, where photosynthesis occurs) and, most importantly, a nucleus, a region surrounded by a double membrane that contains the genetic material (Figure 7.9). A few years later, the development of molecular phylogeny, which infers evolutionary relationships among organisms from their conserved molecules, challenged this structural dichotomy. The comparison of sequences of RNAs from the small ribosomal subunit (SSU-rRNA) of different organisms revealed three, instead of two, major phylogenetic groups. Eukaryotes were one of them, but prokaryotes appeared to be divided in two groups that were as far from one another as they were from eukaryotes. They were initially called 'Eubacteria' and 'Archaebacteria' (Woese and Fox, 1977), and later re-baptized in the so-called domains Bacteria and Archaea (Woese et al., 1990). Molecular biology and biochemistry studies subsequently revealed many fundamental differences between them (Zillig, 1991). Despite the major differences between non-eukaryotic organisms, eukaryotes constitute both, phylogenetically and structurally a distinctive set of life forms. When, and particularly, how they originated is a matter of vivid controversy, as we will try to briefly summarize in the following.

7.3.1 Different hypotheses for the origin of eukaryotes

Due to its simpler cell organization, most researchers believe that some kind of prokaryotic ancestor gave rise to eukaryotes, an idea already implicit in the work of the German evolutionist E. Haeckel (1866). However, the discovery

that prokaryotes are profoundly divided in two groups phylogenetically distinct complicated this view. Furthermore, the first gene comparisons, later corroborated by the analysis of complete genome sequences, uncovered a paradox: eukaryotic genes related to DNA replication, transcription and translation – the basic informational core – resembled archaeal genes, whereas genes involved in energy and carbon metabolism resembled their bacterial counterparts (Rivera et al., 1998). How could this mixed heritage in eukaryotes be explained? A variety of competing models have been put forward for reviews, see (López-García and Moreira, 1999; Martin et al., 2001) (Figure 7.10).

7.3.1.1 *Autogenous models*

The most widely accepted proposal is an elaboration of the traditional prokaryote-to-eukaryote transition, whereby the emergence of the nucleus and most of the other eukaryotic features occurred by complexification of ancestral structures that appeared in a single prokaryotic lineage (Cavalier-Smith, 1987). Since the first attempts to find the origin of the tree of life placed the root along the bacterial branch, implying the sisterhood of archaea and eukaryotes (Gogarten et al., 1989; Iwabe et al., 1989) (see Figure 5.10.A in Chapter 5.7), the prokaryotic lineage from which eukaryotes emerged would be archaeal-like (Woese et al., 1990; Brown and Doolittle, 1997). In this model, the eukaryotic bacterial-like genes would have been imported from mitochondria and chloroplasts to the nucleus, since it has been unambiguously demonstrated that both organelles evolved from bacterial endosymbionts: mitochondria derive from alphaproteobacteria, and chloroplasts from photosynthetic cyanobacteria (Gray and Doolittle, 1982). In a variant, the 'you are what you eat' model, those bacterial genes would come also from bacterial preys (Doolittle, 1998). The nucleus would have formed by invagination of the cell membrane at the same time and by the same mechanism that the endoplasmic reticulum, an internal membrane system for macromolecule transport (Jekely, 2003) (Figure 7.10).

7.3.1.2 *Chimeric models*

To explain the mixed composition of eukaryotic genomes, various hypotheses propose that eukaryotes resulted from the union of two prokaryotic lines, one archaeal and one bacterial (see Figure 5.10.B in Chapter 5.7). Some proposals are relatively simple and suggest a direct fusion or some kind of unspecified symbiosis of one archaeon and one bacterium (Zillig, 1991), either by an engulfment of one archaeon by a bacterium (Lake and Rivera, 1994; Gupta and Golding, 1996) or by a hypothetical descendant of an RNA world (Sogin, 1991) (Figure 7.10). Symbiosis-based models are the more comprehensive of the chimeric proposals. Symbiosis (*living-together*) between different organisms does not imply merely the sum of the parts but may lead to the creation of novel functions and properties as a consequence of gene

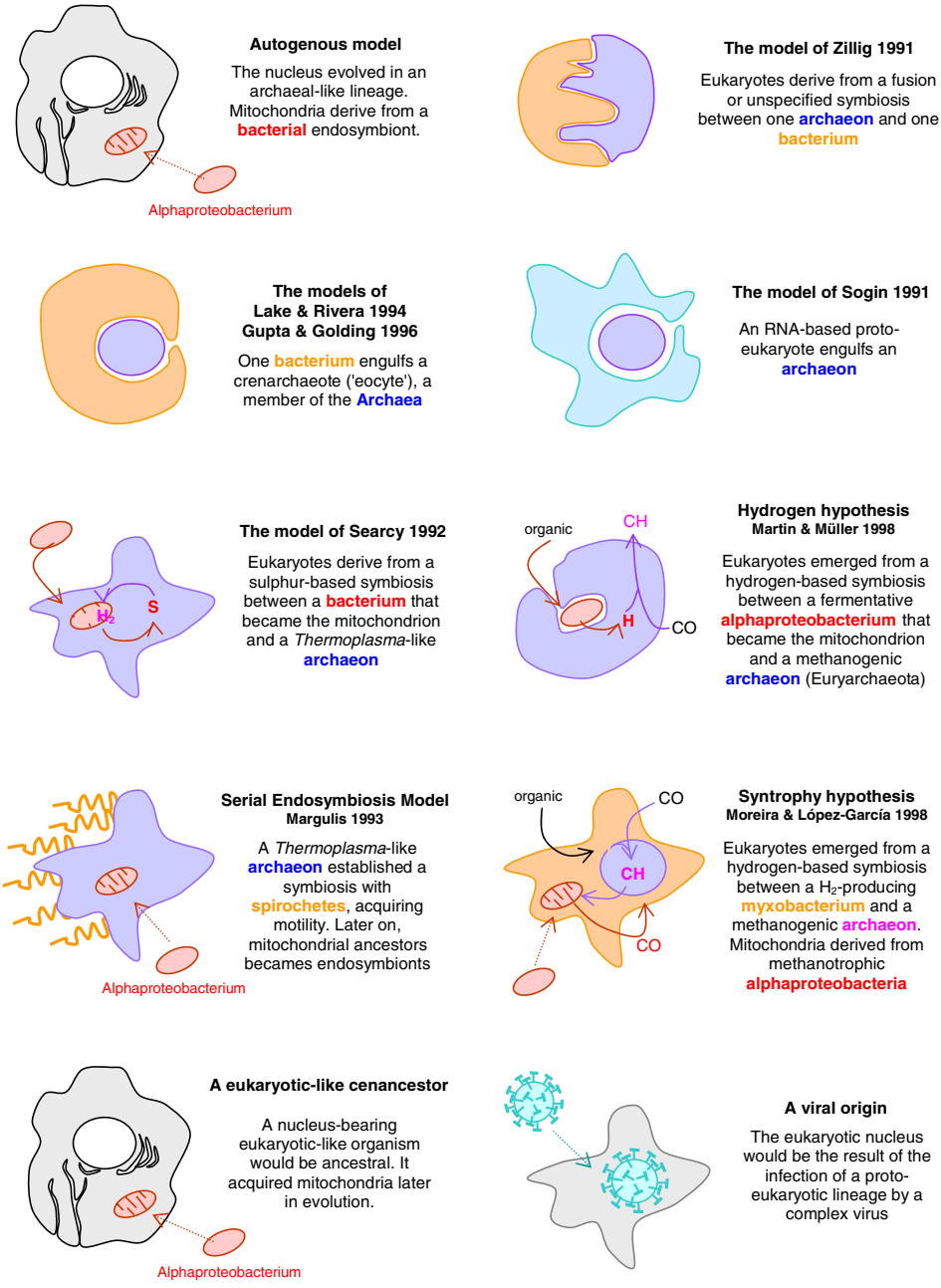

Figure 7.10. Different models for the origin of eukaryotes.

redundancy and increased evolutionary rate (Kirschner and Gerhart, 1998; Margulis and Fester, 1993). Excellent examples are mitochondria and chloroplasts, once bacterial endosymbionts, which have positively contributed to the make-up of contemporary eukaryotes, attesting to the incontestable importance of symbiosis in eukaryotic evolution. Under these models, a long-term symbiotic relationship between one archaeon and one bacterium could have ended up in a novel entity, a eukaryote.

Detailed symbiotic proposals for the origin of eukaryotes, those that explicit a selective advantage for the involved partners, can be classified in two main classes. A first group of models states that eukaryotes stemmed from a symbiosis established between the ancestor of mitochondria (a bacterium) and one archaeon, more precisely one euryarchaeote (Euryarchaeota and Crenarchaeota are the two major branches of Archaea) (Figure 7.10). One hypothesis proposed a symbiosis between a wall-less euryarchaeote (a *Thermoplasma*-like species) able to reduce S_0 to H_2S and a bacterium oxidizing H_2S to S_0. To increase the efficiency of the exchange of sulfur species, the bacterium (the future mitochondrion) became an endosymbiont of the archaeon (Searcy, 1992). The hydrogen hypothesis states that the ancestor of mitochondria, one alphaproteobacterium able to ferment organics liberating H_2, established a symbiosis with a methanogenic archaeon (one euryarchaeote) using H_2 to reduce CO_2 to CH_4. As in the previous case, the bacterium became an endosymbiont of the archaeon and, with time, the mitochondrion (Martin and Muller, 1998). A second group of models envisages that the bacterium involved in the initial eukaryogenetic symbiosis was different from the mitochondrial ancestor, and that mitochondria derived from a second symbiotic event (Figure 7.10). The Serial Endosymbiosis Theory (SET) thus states that a first symbiosis was established between a *Thermoplasma*-like archaeon and spirochetes, which would have conferred motility to the ensemble, becoming eukaryotic flagella. The consortium evolved to form a proto-eukaryotic cell that then acquired the mitochondrial endosymbiont (Margulis, 1981). The syntrophy hypothesis proposes that eukaryotes arose from a symbiosis based on interspecies hydrogen transfer between an ancestral myxobacterium (gliding bacteria with complex developmental cycles belonging to the deltaproteobacteria) and a methanogenic archaeon. The ancestor of mitochondria would be a versatile alphaproteobacterium able, among others, to oxidize the CH_4 produced by the archaeon (Moreira and López-García, 1998; López-García and Moreira, 1999). Whereas in the above-mentioned models the eukaryotic nucleus was formed *de novo* in the cytoplasm of the archaeal host, in the syntrophy hypothesis the nucleus would be a relic of the archaeal partner.

Since the different hypotheses predict that the eukaryotic nucleus should contain genes coming from various specific prokaryotic groups, comparative genomic analysis of the rapidly increasing number of prokaryotic and

eukaryotic complete genome sequences should allow in a next future to corroborate or refute some of these hypotheses.

7.3.1.3 *Other models*

Although most authors favor a prokaryote-to-eukaryote transition, various scientists have argued that prokaryotes are reduced descendants of more complex, eukaryotic-like ancestors already endowed with a nucleus (Bisset, 1973; Reanney, 1974; Poole et al., 1998; Forterre and Philippe, 1999). In this case, the origin of eukaryotes would be in essence the origin of the last common ancestor to extant organisms (see Figure 5.10.D in chapter 5.7) which, later in evolution, acquired mitochondria. The recent discovery of a membranous envelope containing the genetic material and part of the cytoplasm in the Planctomycetales together with their apparent early-branching position in the bacterial tree has led some authors to suggest that they could be intermediates between a eukaryotic ancestor and the rest of bacteria (Fuerst and Webb, 1991; Brochier and Philippe, 2002). However, the analysis of the genome sequence of the planctomycete *Rhodopirellula baltica* does not reveal any particular similarity with eukaryotes (Glockner et al., 2003). Finally, a recent set of models proposes that the eukaryotic nucleus derived from complex viruses related to Poxviruses (Bell, 2001; Takemura, 2001; Villarreal, 2005) (Figure 7.10). Several features of the Poxviruses cell cycle are reminiscent of the eukaryotic nucleus biology. In its original version, the authors of the viral eukaryogenesis theory suggested that the virus at the origin of the nucleus infected a wall-less methanogenic archaeon. Later on, it was proposed that the host was a more primitive cell (even possibly an RNA cell) (Forterre, 2005).

7.3.2 The last common ancestor of contemporary eukaryotes

Be as it may, there is one certitude that is relevant for chronological aspects: modern eukaryotes emerged only after prokaryotes had appeared and diversified. This is the only explanation to the fact that all known eukaryotes have or have had mitochondria, which themselves derive from already quite modified bacteria, the alphaproteobacteria. Eukaryotes lacking apparent mitochondria exist and, in addition, they branched at the base of the eukaryotic tree in initial phylogenetic analyses. For some time, they were thought to be primitive eukaryotes that preceded the mitochondrial acquisition (Sogin, 1991). However, subsequent studies refuted this view. First, several genes of undeniable mitochondrial origin were found in the genomes of these mitochondrial-lacking eukaryotes, suggesting that they harbored these organelles once but lost them, in many cases because of a radical adaptation to a parasitic lifestyle (Simpson and Roger, 2002). Second, the improvement of evolutionary models in phylogenetic analyses together with

the incorporation of many more eukaryotic sequences showed that those lineages had been misplaced to the base of the tree due to methodological artifacts (Simpson and Roger, 2002; Baldauf, 2003). Today, although the eukaryotic tree is not fully resolved, it is widely accepted that the last common ancestor of extant eukaryotes possessed mitochondria. Most scientists also think that eukaryotes suffered a radiation, i.e. they diversified in a very short time span, which would explain the difficulties to determine the relative order of emergence of the major eukaryotic lineages (Philippe et al., 2000). The cause of that sudden radiation is unclear. For some authors it was the acquisition of mitochondria which, providing O_2 respiration, granted a great selective advantage to colonize a variety of new ecological niches (Philippe et al., 2000). In conclusion, eukaryotes *sensu stricto* – i.e. possessing a nucleus, a well-developed cytoskeleton and organelles – evolved after and derived, at least partly, from prokaryotes. But when?

7.3.3 When did eukaryotes appear and diversify?

7.3.3.1 *Fossil record*

There is little agreement as to when the first eukaryotic traces appeared in the fossil record. The oldest traces claimed to be of eukaryotic origin are biochemical: sterane compounds found in 2.7 Ga old kerogenes (Brocks et al., 1999). Steranes are fossil lipids derived from sterols, typically synthesized by eukaryotes. However, this finding is highly controversial because (i) a later contamination of this material by eukaryotes is possible, and (ii) many bacterial groups also synthesize sterols including, at least, methanotrophic bacteria, cyanobacteria, myxobacteria, actinobacteria and planctomycetes (Ourisson et al., 1987; Pearson et al., 2003). The oldest morphological eukaryotic fossils have been claimed to correspond to the coiled, spaghetti-like *Grypania spiralis* (~2 Ga ago) because of their large size (Han and Runnegar, 1992). However, size alone is not a definitive criterion as prokaryotic and eukaryotic sizes overlap (Javaux et al., 2003). The eukaryotic status of *Grypania* is highly discussed, as it may rather simply correspond to large cyanobacterial filaments (Cavalier-Smith, 2002). Surface ornamentation appears to be a more defining criterion since unicellular eukaryotes often display different scales and other surface structures that confer them an idiosyncratic aspect, while prokaryotes lack decoration in individual cells. The oldest decorated fossils, acritarchs, date from 1.5 Ga ago and were identified in the Mesoproterozoic Roper Group (Northern Australia) (Javaux et al., 2001). It is however difficult to relate this fossil group to any of the extant eukaryotic lineages (Javaux et al., 2003). The oldest fossils that have been assigned to a modern eukaryotic lineage correspond to *Bangiomorpha pubescens*, claimed to belong to red algae (Butterfield et al., 1990), and dated

from 723 Ma to 1.267 Ga ago, yet certainly closer to the latter bound (Butterfield, 2001). In summary, although there is reasonable morphological evidence suggesting that eukaryotes have developed by 1.5 Ga ago, their traces are sparse in rocks older than 1 Ga; Cavalier-Smith (2002) has even proposed that eukaryotes appeared in the fossil record only relatively recently, ~850 Ma ago, and that all older eukaryotic-like fossils corresponded to extinct groups of morphologically complex bacteria.

7.3.3.2 *Molecular dating*

Due to the scarcity of the eukaryote fossil record, the chronology of their origin and diversification has always been difficult to establish (Knoll, 2003). Comparative analysis of genomic data – homologous DNA, RNA, and protein sequences sampled from eukaryotic genomes – provided an alternative approach to reconstruct the history of life on Earth by (inter)connecting geological, paleontological, and biological information (Benner et al., 2002). Biomacromolecular sequences retain information about past history, and their degree of divergence among organisms has been correlated to the time of separation from their last recent common ancestor: the greater the number of differences between genomes or proteomes, the deeper the age of the split between the corresponding species. This hypothesis of a molecular clock ticking in biomolecules, i.e. the relative constancy of molecular evolutionary rate over time (Zuckerkandl and Pauling, 1965), is presented and discussed in Chapter 2.4 (*Biological chronometers: the molecular clocks*).

Several molecular dating studies attempted to evaluate divergence times of the major lineages of eukaryotes, with special focus on plants, animals, and fungi (Table 7.1). A striking concern about these independent multigene studies is the large range of estimates proposed for eukaryotic divergence times. For example, the dichotomy between animals and fungi is supposed to have occurred between 1.513 Ga ago (beginning of the Mesoproterozoic: Hedges et al., 2004) and 984 Ma ago (beginning of the Neoproterozoic; Douzery et al., 2004). Deeper divergence times corresponding to the split between unicellular (protists) and multicellular eukaryotes also display a wide range of estimates, from 1.545 to 2.309 Ga ago (Table 7.1). It would be desirable to directly compare all these results, but the different dating approaches (global vs. relaxed molecular clocks), genes and proteins (variable number of sites sampled from nuclear and plastid compartments), paleontological calibrations (from one to six, with or without incorporation of fossil record uncertainty), and taxon samplings make this difficult. However, more reliable estimates are expected to be inferred from larger data sets (typically more than 100 markers in order to reduce stochastic errors), under relaxed molecular clocks that are not hampered by the detection of constant rate sequences, and with the aid of several independent primary calibrations. In this context, the age of the dichotomy between

TABLE 7.1.
Molecular dating of the divergence of the major groups of eukaryotes compiled from several references

Splits	Ages (Ma)	Markers	Clocks	Calibrations	References
Animals + Fungi	984 (SD ± 65)	129 proteins 30,399 aa	Relaxed (rate auto correlation)	6 constraints (P)	Douzery et al. (2004)
Animals + Fungi	1513 (SE ± 66)	69–92 proteins 31,362 aa	Global/Relaxed	1 constraint (P) 3 points (S)	Hedges et al. (2004)
Plants–Animals–Fungi	1215–1272 (–)	64 proteins 25,000 aa	Global	6 points (P)	Feng et al. (1997)
Plants–Animals–Fungi	1576 (SD ± 88)	38 proteins 22,888 aa	Global	1 point (P)	Wang et al. (1999)
Plants–Animals–Fungi	1392 (SE ± 256)	11 proteins 3310 aa	Global	1 point (P)	Nei et al. (2001)
Plants/[Animals + Fungi]	1085 (SD ± 79)	129 proteins 30,399 aa	Relaxed (rate auto correlation)	6 constraints (P)	Douzery et al. (2004)
Plants/[Animals + Fungi]	1609 (SE ± 60)	99–143 proteins 60,274 aa	Global/Relaxed	1 constraint (P) 3 points (S)	Hedges et al. (2004)
Photosynthetic eukaryotes/Animals	> 1558 (1531–1602)	6 plastid genes 7111 nt	Relaxed (penalized)	6 constraints (P)	Yoon et al. (2004)
Protists/Plants–Animals–Fungi	1545 (–)	64 proteins 25,000 aa	Global	6 points (P)	Feng et al. (1997)
Protists/Plants–Animals–Fungi	1717 (SE ± 349)	11 proteins 3310 aa	Global	1 point (P)	Nei et al. (2001)

TABLE 7.1.
Continued

Splits	Ages (Ma)	Markers	Clocks	Calibrations	References
Giardia/Other eukaryotes	2230 (SE ± 120)	17 proteins –	Global	3 points (S)	Hedges et al. (2001)
Giardia/Plants–Animals–Fungi	2309 (SE ± 194)	28–32 proteins 11,251 aa	Global/Relaxed	1 constraint (P) 3 points (S)	Hedges et al. (2004)

Estimates of divergence ages and their uncertainties (when available) are given in million years ago. The number of markers, the type of molecular clock methodology, and the number of fossil calibrations used for the dating are also given. Calibrations might be primary (P, derived from fossils) or secondary (S, derived from molecules), and incorporate paleontological uncertainty (constraints) or not (points). Abbreviations – nt: nucleotide sites; aa: amino acid sites; SD: standard deviation; SE: standard error.

plants (i.e., photosynthetic eukaryotes) and animals + fungi would be closer to 1.085 Ga ago (Douzery et al., 2004) than to 1.609 Ga ago (Hedges et al., 2004). This suggests that primary plastids resulting from the endosymbiosis of a free-living cyanobacterium appeared at the end of the Mesoproterozoic some 1.1 Ga ago.

The postulated anoxic and sulfidic redox status of oceans until 1 Ga might have limited the rise of photosynthetic eukaryotes (Anbar and Knoll, 2002), whereas cytoskeletal and ecological prerequisites for their diversification were already established some 1.5 Ga ago (Javaux et al., 2001). The more oxygenic environments of the Neoproterozoic (1 Ga to 540 Ma) could possibly have triggered the diversification of the major eukaryotic lineages, culminating with the seemingly abrupt appearance of animals in the Cambrian explosion (Conway Morris, 2000).

7.4. The Neoproterozoic–Cambrian Transition (~1000 to 542 Ma)

Philippe Claeys

The end of the Proterozoic (Neoproterozoic ~1000 to 542 Ma) corresponds to a period of major global changes most likely initiated by the break-up of the supercontinent Rodania around 750 Ma ago (Kah and Bartley, 2001). Three widespread and severe glaciation events occur during the Neoproterozoic: the Sturtian (~710 to 725 Ma), the Marinoan (~635 to 600 Ma), and Gaskiers (~580 Ma) (Figure 7.11). These events are identified based on isotopic profiles ($^{13}C/^{12}C$ and $^{87}Sr/^{86}Sr$) and the repetitive accumulation of thick packages of glacial sediments, recognized worldwide. These tillites (or diamictites) are commonly covered by distinctive cap carbonates, which, curiously, almost certainly precipitated inorganically under warm-water conditions. The Sturtian and Marinoan events were probably the most extreme glaciations recorded on Earth. Evans (2000) provided paleomagnetic evidence for the presence of glaciers at sea level within 10 ° from the Neoproterozoic equator. Hoffman et al. (1998a) proposed that the Sturtian and Marinoan were global glaciations covering the entire planet, commonly referred to as the *Snowball Earth* hypothesis. The thick ice cover (up to 1 km) implies a drastic reduction in photosynthesis and a collapse of biological productivity that best explains the intensity of the negative carbon isotopic anomalies (up to $-14‰$ in surface ocean $\partial^{13}C$) recorded in carbonates bracketing glacial sediments (Hoffman et al., 1998a). The presence of continental ice inhibited silicate weathering, another CO_2 sink, leading to its accumulation in the atmosphere. Outgassing by subaerial volcanoes contributed further to the increase of CO_2 in the atmosphere throughout the icehouse period. The glacial conditions ended abruptly when greenhouse gas concentrations

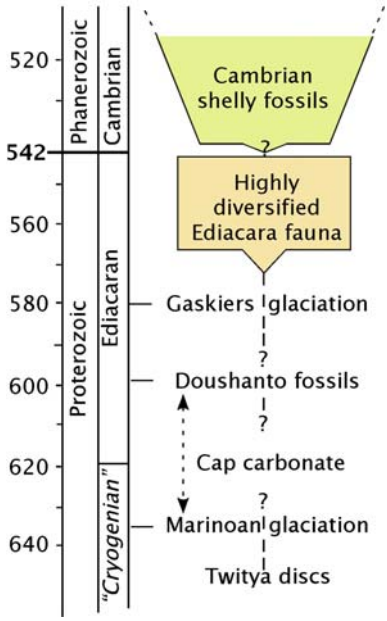

Fig. 7.11. Schematic stratigraphy of the Proterozoic–Cambrian transition.

became high enough (~0.12 bars of CO_2 in the atmosphere) to overcome the albedo effect, causing rapid melting of the ice and subsequent precipitation of warm-water carbonates (Hoffman et al., 1998a). On such a fully glaciated Earth, extraterrestrial material would accumulate on and within the ice. Based on Ir flux measured at the base of the cap carbonates, the duration of the Marinoan glaciation is estimated around 12 Ma (Bodiselitsch et al., 2005). The Snowball hypothesis is currently subject to lively debates (see Jenkins and Scotese, 1998; Christie-Blick et al., 1999; and replies by Hoffman et al., 1998b; Hoffman and Schrag, 1999 for example) focusing on the initiation and termination of the glaciations, as well as the global extent and average thickness of the ice. Some authors consider that (large) parts of the ocean must have remained ice-free, forming a sanctuary for marine organisms where photosynthesis could continue. This alternative view is called the ''Slushball Earth'' hypothesis (Hyde et al., 2000; Crowley et al., 2001).

Because of the absence of skeletonized fossils, Proterozoic lithostratigraphic units are often difficult to correlate precisely and remain subdivided in broad periods defined essentially on the basis of the chronometric ages obtained by isotopic dating of specific but sporadic layers. Recently, a new stratigraphic period: the Ediacaran (Knoll et al., 2004), defined in analogy with its Phanerozoic counterparts, has been approved by the International Union of Geosciences (IUGS) to represent the most recent part of the Proterozoic. The top of the Ediacaran corresponds to the well-defined base of the

Cambrian, dated at 542 Ma (Gradstein et al., 2004). Its base is placed in a distinct carbonate layer that overlies sediment deposited by the Marinoan glaciation in the Flinders Ranges of South Australia (Figure 7.11). The base of the Ediacaran is not precisely dated. It is younger than an U-Pb date of 635.5 ± 1.2 Ma measured on zircons from within the glacial diamictites of Namibia and older than a Pb–Pb date of 599 ± 4 Ma obtained on post-glacial phosphorites in China (Barfod et al., 2002; Hoffman et al., 2004; Knoll et al., 2004). In term of event stratigraphy, the Ediacaran is bounded above by the rapid diversification of shelly organisms and below by the Marinoan ice age of global extent (Knoll et al., 2004).

The Ediacaran period is characterized by the presence of the traces of soft-body organisms fossilized as impression on sandstone beds or less commonly on ash-layers (Figure 7.12). Such fossils lacking a mineralized shell (or skeleton) differ significantly from their Phanerozoic counterparts. Named after the remarkable collection recognized in South Australia, almost 60 years ago, the Ediacara fauna occurs worldwide (30 localities on 5 continents, Narbonne, 1998). At most localities, the preserved fossils attest of highly diversified and sophisticated organisms displaying a great variety of

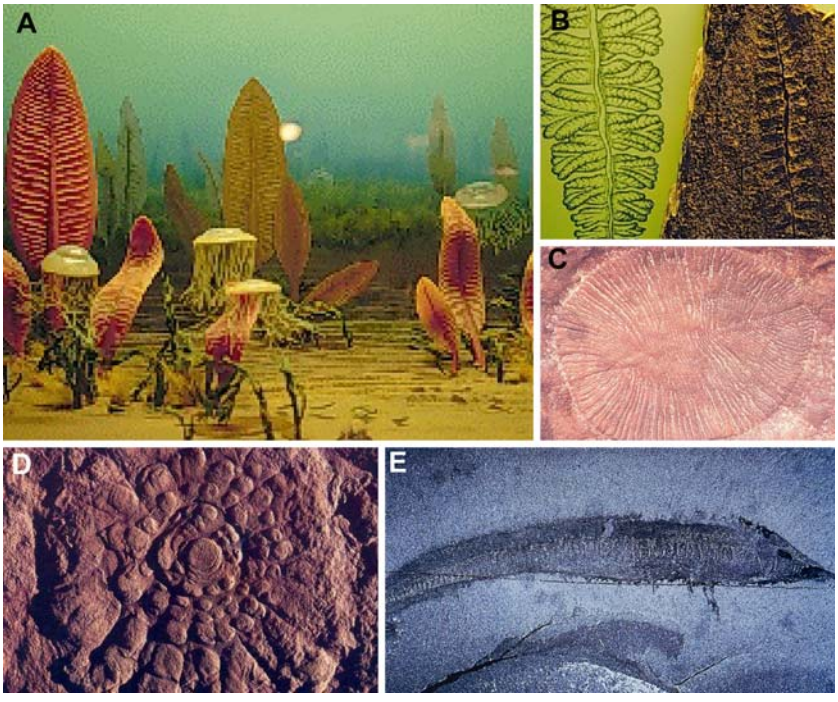

Figure 7.12. Ediacaran fauna. (A) reconstruction of the Ediacaran environment; (B) Chania (Cnidaria?); (C) Dickensonia (worm? cnidaria?); (D) Mawsonites (medusa); (E) Pikaia, Burgess Pass (first known chordate).

shapes and sizes. This complex fauna forms a biological transition between the modern shelly organisms of the Phanerozoic and the essentially microbial communities of the Proterozoic, dominated by prokaryotes and microbial eukaryotes including algae. Although, the first Eukaryotes probably appeared between 2.7 and 1.8 Ga ago, they do not seem to have been abundant or diversified until the end of the Proterozoic (Knoll, 2003). Javaux et al. (2004) reported the presence of a moderate diversity of probable eukaryotic remains, among a fauna rich in protistan microfossils in carbonaceous shales dated between 1.5 and 1.4 Ga from Australia.

Either Snowball or Slushball, both scenarios seem capable to strongly influence the evolution of life. Ediacara fauna diversified within a few million years after the last Neoproterozoic glacial event (Narbonne, 2005). The less severe glaciation at the end of the Ordovician caused one of the major mass extinctions of the Phanerozoic (Sheehan et al., 1996). Although not clearly linked to extinction, these Neoproterozoic glaciations followed by greenhouse conditions, must have been harsh selection factors, possibly triggering in their aftermath the radiation of Ediacaran organisms. An issue that remains unclear is the role of the rising oxygen concentrations (Knoll, 2003). The level of oxygen necessary for the development of large-scale metazoans is estimated between 1% and 10% of that of the present day (Knoll and Holland, 1994). Post-glacial oxygenation may also have favored the diversification of the Ediacara organisms (Narbonne, 2005).

The characteristic Ediacara biota clearly marks the first appearance on Earth of large, complex and highly diversified communities. These fossil assemblages contain radial and bilateral organisms constituting, perhaps the root-stock of the Cambrian radiation, possible life-forms belonging to other, now extinct, eukaryotic phyla or kingdoms, and/or what appear as ''failed experiments'' in animal evolution (see Narbonne, 2005 for a detailed review). The stratigraphic distribution of the Ediacara organisms is rather well constrained. The Twitya formation of northwestern Canada contains, just below the Marinoan tillites, a poorly diversified assemblage of ''Twitya discs'' considered perhaps as the oldest known Ediacara-type fossils (Hofmann et al., 1990). Possible bilaterian eggs and embryos as well as fossilized cnidarian may be present in the Doushantuo formation in China (Xiao et al., 1998), which is dated by Pb–Pb and Lu–Hf on phosphates between 599 and 584 Ma (Barfod et al., 2002). These still enigmatic fossils clearly predate the major radiation of Ediacara organisms. Conway Morris (1998) recognizes rare Ediacara survivors among the Cambrian Burgess shale fauna. Nevertheless, the typical, highly diversified Ediacara fauna is restricted to a well-constrained stratigraphic interval between ~575 and 542 Ma (Figure 7.11); starting just above the youngest glacial deposits (Gaskiers) of the Neoproterozoic and extending to the very base of the Cambrian (Narbonne, 2005).

The typical Ediacara fossils are commonly a few cm to 10 cm in size but some giant forms reach more than a meter in length. They display a great range of shapes such as disks, fronds or segmented morphologies, somewhat reminiscent to those of modern organisms (Narbonne, 2005). Other forms are highly unusual and completely unique to the Ediacaran fauna. Ediacara fossils lived on soft and muddy seafloor and are best preserved when rapidly buried by a coarse sedimentary event such as the deposition of turbidites, or ash layers. In the best-preserved sections, the abundance of organisms is comparable to that found in equivalent modern seafloor communities (Narbonne, 2005).

Most of the Ediacara organisms cluster in 3 assemblages (see Narbonne, 2005 for a detailed discussion): (1) the Avalon assemblage (575–560 Ma), characterized by apparently more primitive and bizarre shapes, found in deep-water settings, (2) the White Sea assemblage (560–542 Ma), more diverse, living in shallow-water and composed of segmented, disk, and front morphologies, with some bilaterian organisms capable of mobility, (3) the Nama assemblage (549–542 Ma), also of shallow-water but marked by the presence of some early calcified metazoans. The relationships between Ediacara biota and modern organisms were rather controversial during the mid-1980s and early 1990's. Early work based on morphologies had advocated similarities with the jellyfishes. For Seilacher (1992), Ediacara fauna differ drastically from modern organisms and represent an extinct Kingdom of life, which he called Vendobionta. Today, there seems to be an agreement that ancestors of radial phyla such as Porifera and Cnidaria dominated the Ediacara fauna (Narbonne, 2005). McCaffrey et al. (1994) reported the presence of sponge biomarkers in Neoproterozoic hydrocarbon deposits. Other body fossils with evident bilateral symmetry and segmentation point to possible ancestors of the phyla Arthropoda, Mollusca, Annelida and Echinodermata (Budd and Jensen, 2000). A fraction of Ediacara taxa appears unrelated to modern organisms and may represent extinct phyla (Knoll, 2003).

The transition between the Ediacara fauna and the Cambrian shelly fossils is not clearly understood. The apparently abrupt disappearance of the Ediacara fossils, just below the base of the Cambrian could be linked to a major anoxic event (Kimura and Watanabe, 2001) or to the rise of widespread predation among organisms (Bengtson and Yue, 1992). The subsequent ''Cambrian Explosion of life'' reflects again a major diversification of biosphere and, because of the appearance of a hard shell a much better preservation of the fossil remains. The small shelly fossils either made of carbonates or phosphates are widespread in the very basal Cambrian, preceding the rich and highly diversified fauna such as that preserved later in the Burgess shales (Conway Morris, 1998) or other fossiliferous beds. All present

day phyla, along with a few enigmatic taxa, are already present at this level of the Cambrian. No new phylum will emerge in the next 500 Ma.

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