Contemporary research instead has firmly placed hydrogenosomes in the evolutionary lineage of mitochondria.[37] They have lost the apparatus of oxidative phosphorylation but retain that for protein import. As a rule hydrogenosomes lack a genome but one at least, from a ciliate that flourishes in the hindgut of termites, features a residual genome that clearly resembles mitochondrial ones. Hydrogenosomes have apparently arisen independently on many occasions, as eukaryotic cells explored diverse anaerobic niches. Nor are hydrogenosomes the end of the line. A number of anaerobic protists, including such familiar parasites as *Giardia* and *Entamoeba,* lack both mitochondria and hydrogenosomes but contain certain genes normally associated with mitchondria. Their protein products were found to be localized in minute membrane-bound organelles, previously overlooked, that are now called mitosomes.[38] Mitosomes lack genomes and produce neither ATP nor hydrogen gas, but do import proteins by the canonical pathway. They fuel their activities with ATP imported from the cytoplasm. Just what functions mitosomes perform is still uncertain; the only known one is the synthesis of iron sulfide clusters, an important cellular process required for the maturation of diverse proteins, normally localized in mitochondria. Mitochondria, hydrogenosomes, and mitosomes are evidently members of an extended family that is sure to grow as more anaerobic protists are examined in detail. At the present time, the only features common to them all appear to be the biosynthesis of iron sulfide clusters and the possession of two membranes, all of bacterial ancestry. It will be interesting to see whether any eukaryotic cells have dispensed with their mitochondrial legacy altogether.

Reductive evolution is a feature of the plastid lineage as well.[39] Photosynthesis has, of course, been lost many times in representatives of all the green phyla, but most of these still feature plastids that supply their host cell with fatty acids, isoprenoids, and the tetrapyrrole compounds that are essential elements of electron carriers. In addition, some lineages that lack overt plastids but were suspected to be derived from plastid-bearing ancestors, have recently been found to contain cryptic plastids. A case in point is the apicoplast of apicomplexan parasites, which include the causative agent of malaria. Here again, the functions of this residual organelle include the provision of fatty acids, isoprenoids, and heme. Can plastids be lost altogether? The issue is important for understanding the phylogeny of several prominent taxa including ciliates, which show no trace of a plastid but may perhaps be descended from plastid-bearing ancestors.[40] If so, did any of their genes survive, and what became of their membranes?

CHAPTER NINE

Reading the Rocks

What We Can Infer from Geology

There is something about walking, and walking long distances, which can lift you out of the cares of the modern world and put you in touch with an older, quieter way of life. — Edwin Mullins, *The Pilgrimage to Santiago*

Snapshots of Two Billion Years Ago
Whispers from the Beginning
In Search of the First Eukaryotes
A Change in the Air
A Timeline for Cell Evolution

Lake O'Hara, a favorite haunt of all who love the Canadian Rockies, is nestled at the head of a long valley in the heart of Yoho National Park. A gated road gives access to the water and the lodge, but past those all ways lead up, steeply up into the alpine. My choice for today is the track that climbs to Lake Opabin, well beyond the timberline; on the far side, above the glacier, Mount Hungabee soars another 1,200 meters to touch the clouds at nearly 3,500 meters. It's a great place to sit, doze a little, and think about time.

The rocks around here are paleozoic marine sediments, laid down in shallow water and occasionally inlaid with fossils; the trilobite beds of Mount Stephen are just a few miles away, the Burgess Shale not much further. How long would it take to accumulate a slab the thickness of Mount Hungabee? Well, assuming a net deposition rate of 1 millimeter per century and neglecting losses due to erosion, 1,200 meters call for 120 million years, a fair span even as geologists measure time (but still only a quarter of the time elapsed since these rocks were laid down). To reach the era of cell evolution, say 1.2 billion years ago, we would have to pile 10 Hungabees, one on top of another, and that only gets us one-third of the way to the beginnings of life. All the metaphors and verbal gymnastics we contrive to make the remote past feel more homelike wither before the sheer immensity of geological time.

It does help some to divide eternity into smaller segments, and these have traditionally drawn on the fossil record to supply a timeline (fig. 9.1). By convention, the time of visible life, from the present to the beginning of the Cambrian era 543 million years ago, is designated the Phanerozoic eon. Earlier times are informally labeled "Precambrian" and divided into the Proterozoic eon (Greek for "earlier life"), 2.5 billion to 543 million years ago; the Archean eon, 4 to 2.5 billion years ago;[1] and the Hadean eon, the time of earth's accretion, which began about 4.5 billion years ago. The Proterozoic was the time when microbes flourished, diversified, and ruled alone; only at the very end of that eon did the first multicellular organisms make an appearance. Life must have originated during the Archean eon, which is therefore of particular interest to students of cell evolution; unfortunately, this is also the epoch whose life left the least and most ambiguous remains.

Sedimentary rocks of great antiquity still exist, but very few escaped metamorphosis by burial, heating, and compression. Not surprisingly, the fossil record of early life is sparse, spotty, hard to read, and full of gaps. But it is of the utmost importance, for geology is our only source of information that does not depend on contemporary organisms. And for all its deficiencies, the testimony of the rocks is unambiguous on the central point: the earth has hosted life, specifically microbial life, for almost as long as physical circumstances allowed, at least 3.4 billion years. All through its long-drawn history, life evolved in concert with its physical environment, shaped by geology and reshaping the earth in turn. The works of life include gigantic limestone reefs made up of the shells of innumerable marine protists; deposits of major commercial importance including oil, coal, sulfur, and iron; and most significantly, all the atmosphere's oxygen. That in turn altered the composition of the ocean, changed the pattern of weathering, and brought on global ice ages. We inhabit a living planet whose fortunes have been interwoven with the evolution of life.

This chapter does not pretend to offer a systematic introduction to early life or planetary history. That has been done most enjoyably by Andrew Knoll, whose fingerprints can be spotted all over this chapter;[2] more tech-

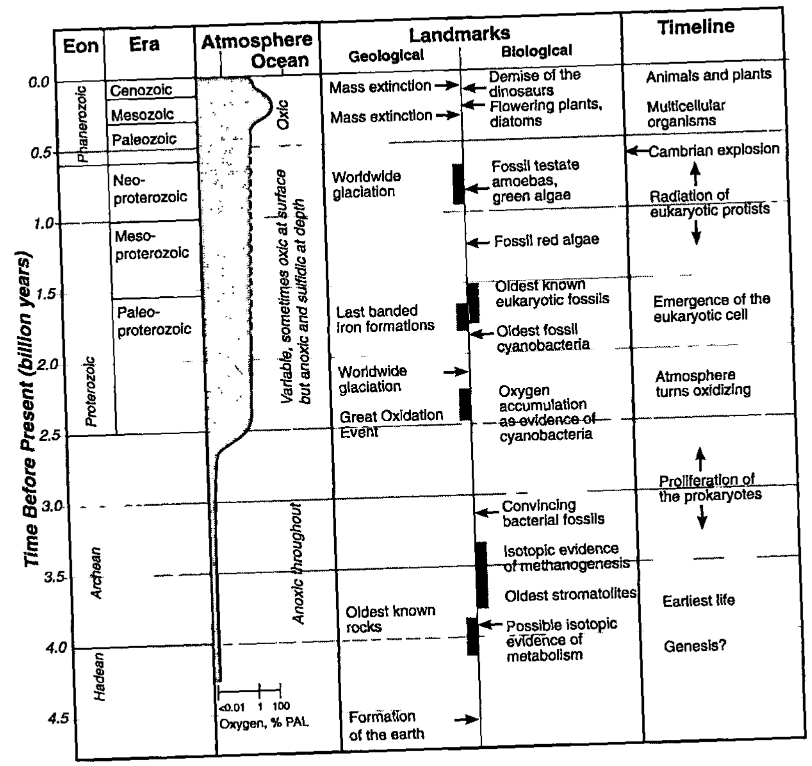

FIGURE 9.1. A timeline for cell evolution. (After Brocks and Banfield 2009.)

nical treatments are available in several symposia and a current textbook of astrobiology.[3] Nick Lane has written a lively yet scholarly introduction to oxygen and the way it shaped the world we now take for granted.[4] Suffice it here to consider what we have learned from paleontology about the earliest stages of biological evolution; readers may find figure 9.1 helpful in keeping track of the argument.

Snapshots of Two Billion Years Ago

Elso Barghoorn first made his name as a paleobotanist working on coal and was drawn into the search for fossil evidence of microbial life by collaboration with a field geologist, Stanley Tyler. Then, in 1954, the gods smiled on them: they reported the discovery of fossilized bacteria in the

Gunflint formation on the northern shore of Lake Superior and instantly quadrupled the length of life's known history. The minute fossils are embedded in a matrix of chert, commonly known as flint, a dense, hard, and fine-grained sedimentary rock composed almost entirely of interlocking silica crystals. Chert takes a sharp edge (hence its popularity with stone-age toolmakers) and can be cut into thin slices suitable for examination under a high-powered microscope. The Gunflint cherts, deposited some 1.9 billion years ago in a shallow tidal flat, host an abundant and varied assemblage of bacterial forms including cocci, chains, and filaments (fig. 9.2a). Among them are thin tubes coated with iron oxide that resemble contemporary iron-loving bacteria. The fossils are often trapped among the laminations of banded structures called stromatolites (fig. 9.2b), which are thought to represent fossilized microbial mats built up over hundreds of generations to macroscopic size.[5]

Cherts and shales dated between 2.1 billion and 1.8 billion years ago are relatively common, and many harbor well-preserved microfossils. Specific identification is seldom possible, but cherts from the Belcher Islands of the Canadian Arctic, Siberia, and elsewhere contain organisms that seem identical to modern cyanobacteria[6] (see also fig. 9.2c.) This identification is reinforced by the occasional presence of large specialized cells that look just like the resting bodies of cyanobacteria, such as *Anabaena*. These again are found tucked into the fabric of stromatolites, just like ones built by cyanobacteria to this day. Chemical biomarkers tell the same story: organic matter extracted from other cherts contains 2-methylhopanes, products of the degradation of hopanoids characteristic of cyanobacteria and seldom produced by other bacteria.[7] To be sure, neither cell morphology nor biomarkers document metabolic patterns, especially not the all-important capacity for oxygen-producing photosynthesis. Nevertheless, taking the findings at face value, it seems almost certain that by two billion years ago cyanobacteria had evolved their special mode of photosynthesis, and much of their present-day morphological diversity. The capacity for nitrogen fixation also seems to go back at least that far.[8] All these fossils are clearly prokaryotic; rocks of the early Proterozoic have not yielded fossils that can be convincingly interpreted as eukaryotic.

The fly in the ointment is, of course, convergent evolution. Is it not possible that early prokaryotes quite unrelated to today's cyanobacteria evolved deceptively similar morphology because they inhabited similar environments, or even acquired those structures by lateral gene transfer? Possible, but not likely. Resting bodies of this sort are found today only

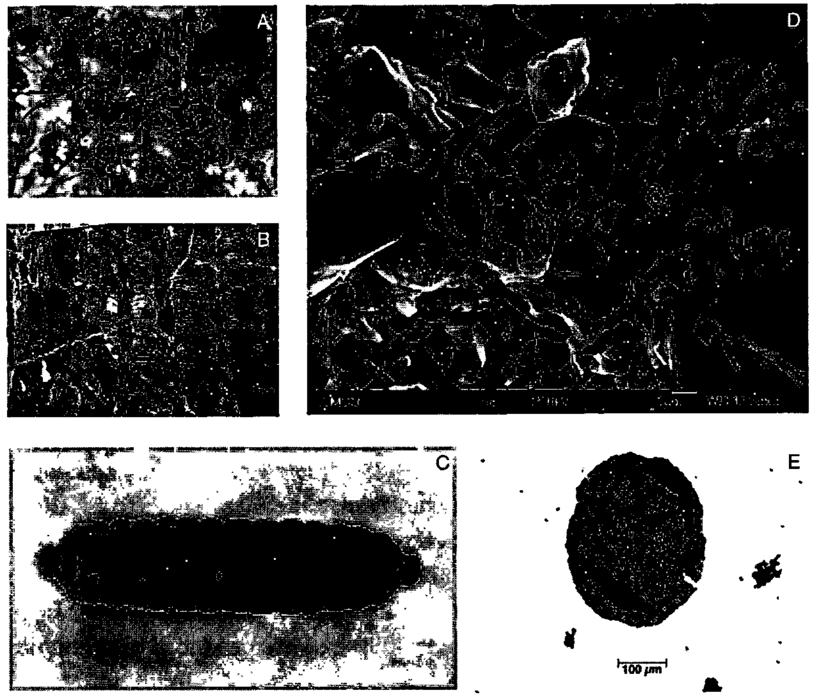

FIGURE 9.2. Proterozoic and Archean fossils. (a) A slice of Gunflint chert, jam-packed with bacterial microfossils (Knoll 2003, courtesy of the author). (b) Stromatolites in Gunflint chert. Each column is about 1 inch wide (Knoll 2003, courtesy of the author). (c) Filamentous cyanobacterium preserved in Siberian chert, 1.5 million years old (Knoll 2003, courtesy of the author). (d). Archean bacteria, about 2.7 billion years old (Westall 2005). The letters identify rod-shaped bacteria (R), cocci (C), and filaments (F). (© 2006, The Geological Society of America, Inc. [GSA]. All rights reserved.) (e). Archean acritarch of uncertain affinities, 3.2 billion years old (Javaux 2010, courtesy of the author, with permission of Nature Publishing Group).

among cyanobacteria, and on their most recent branches at that.[9] Were they products of convergent evolution or subject to transfer, one would expect them to be scattered around the microbial tree. The implication is that cyanobacteria, ancestral to those of today and morphologically as well as physiologically similar, flourished more than two billion years ago. If we accept that argument, at least provisionally, two further implications follow. One is that lateral gene transfer, however common, is not so rampant as to erase lines of descent over two billion years; the other is that cyanobacteria exhibit a degree of evolutionary stasis that puts to shame those conventional paragons of conservatism, horseshoe crabs and brachiopods.

Did those microbial communities of the early Proterozoic eon include members of the Archaea? The question is critical, because the choice between two major versions of the tree of life hinges on whether Archaea came on stage early or late (chapter 4). Unfortunately, neither morphology nor biomarkers can specifically identify the fossil remains of Archaea. The assertion that Archaea were indeed present, and in fact enjoyed great prominence during the later Archean and early Proterozoic eons (say, 2.5 billion years ago) rests on a subtle geophysical clue: the C^{13}/C^{12} ratio of organic material formed at that time.[10] Very briefly, enzymatic reactions commonly discriminate between the carbon isotopes, generally preferring C^{12}. Fixation of atmospheric carbon dioxide by living organisms therefore produces organic matter that is relatively depleted in C^{13}, when compared with carbonate rock produced by inorganic reactions. The effect is especially pronounced for methanogenesis, whose products can therefore be distinguished from those of photosynthesis. The discovery that organic material extracted from certain Archean sedimentary rocks is strongly depleted in C^{13} is taken as evidence that they were produced by methanogenic Archaea.

In many respects, the world of the Gunflint and Belcher microfloras resembled our own. Geologists believe that the ocean was salty, much as it is today, and its temperature about the same or somewhat warmer. Tectonic processes were underway, and there is evidence for at least one worldwide glaciation about 2.2 billion years ago. The great nutrient cycles of carbon, sulfur, and nitrogen were in operation, including nitrogen fixation, though at a slower pace because biological productivity was far lower than at present.[11] The most conspicuous difference between that world and our own is that the Gunflint atmosphere was still very low in oxygen. The strongest evidence comes from a class of iron-rich rocks, the banded iron formations, which can only form in the absence of oxygen. Banded iron formations are plentiful in Archean rocks but then peter out; some of the youngest were laid down in Gunflint times. Incidentally, the prominence of iron-loving bacteria in the Gunflint chert implies that the water was rich in iron, a circumstance that again requires the absence of oxygen. The geological indicators suggest that, by two billion years ago the oxygen level had begun to rise, but was still no higher than 1 percent of the contemporary one. That should not be taken to mean that there could not have been organisms that respired oxygen. Within the lamina of stromatolites constructed by cyanobacteria, oxygen levels may well have been higher. Besides, it now appears that some bacteria, including the familiar *E. coli*, can grow (very slowly) on nanomolar levels of oxygen.[12] So there was something new in the air of Gunflint times, the transformation of the atmosphere had begun, but the oxygen-rich air that we take for granted today still lay far in the future.

Whispers from the Beginning

As we delve deeper into the remote past sedimentary rocks that have escaped destructive metamorphosis become ever harder to find, and truly ancient ones are very rare indeed. Only three localities take us beyond 3 billion years ago. The most productive have been cherts and shales from the Pilbara Hills in northwestern Australia and the Greenstone belt of Barberton Mountain Land in South Africa. Both fall well into the Archean, 3.5 to 3 billion years ago; they are geologically quite similar and may have once been parts of a single province that was severed by tectonic forces. Both have been subjected to heating and compression, sufficient to degrade fossil remains but not to destroy them altogether. The Isua and Akilia formations of southwestern Greenland are substantially older, 3.7 to 3.8 billion years, but have suffered drastic alteration. Some authorities claim that they nevertheless hold isotopic evidence for the existence of microbial life in the early Archean, others dismiss the findings and even question whether those were sedimentary rocks to begin with.[13] As far as we know, all earlier rocks have fallen victim to tectonic mayhem, leaving us without any geological traces of the origin of life.

Despite the limitations, there is general agreement that microbes flourished as far back as 3.4 billion years ago, and probably well before that. The evidence is of the same nature as that which testifies to the Proterozoic biosphere, but much less abundant; relics are less well preserved, and all conclusions are hedged with caveats.[14] The clearest evidence takes the form of stromatolites, and while similar objects can sometimes be produced by inorganic processes, the majority of Archean stromatolites are almost certainly genuine fossils.[15] Microfossils that look in every respect like bacterial cells, including cocci, rods, and filaments (fig. 9.2d) have been retrieved from several locations, including some that may have been part of a hydrothermal vent. Curiously, such fossils are seldom found embedded in stromatolites. Skeptics point out that inorganic processes