The Role of Marine Biota in the Functioning of the Biosphere

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CHAPTER 3

HOW CYANOBACTERIA MADE PLANET EARTH HABITABLE (FOR HUMANS)

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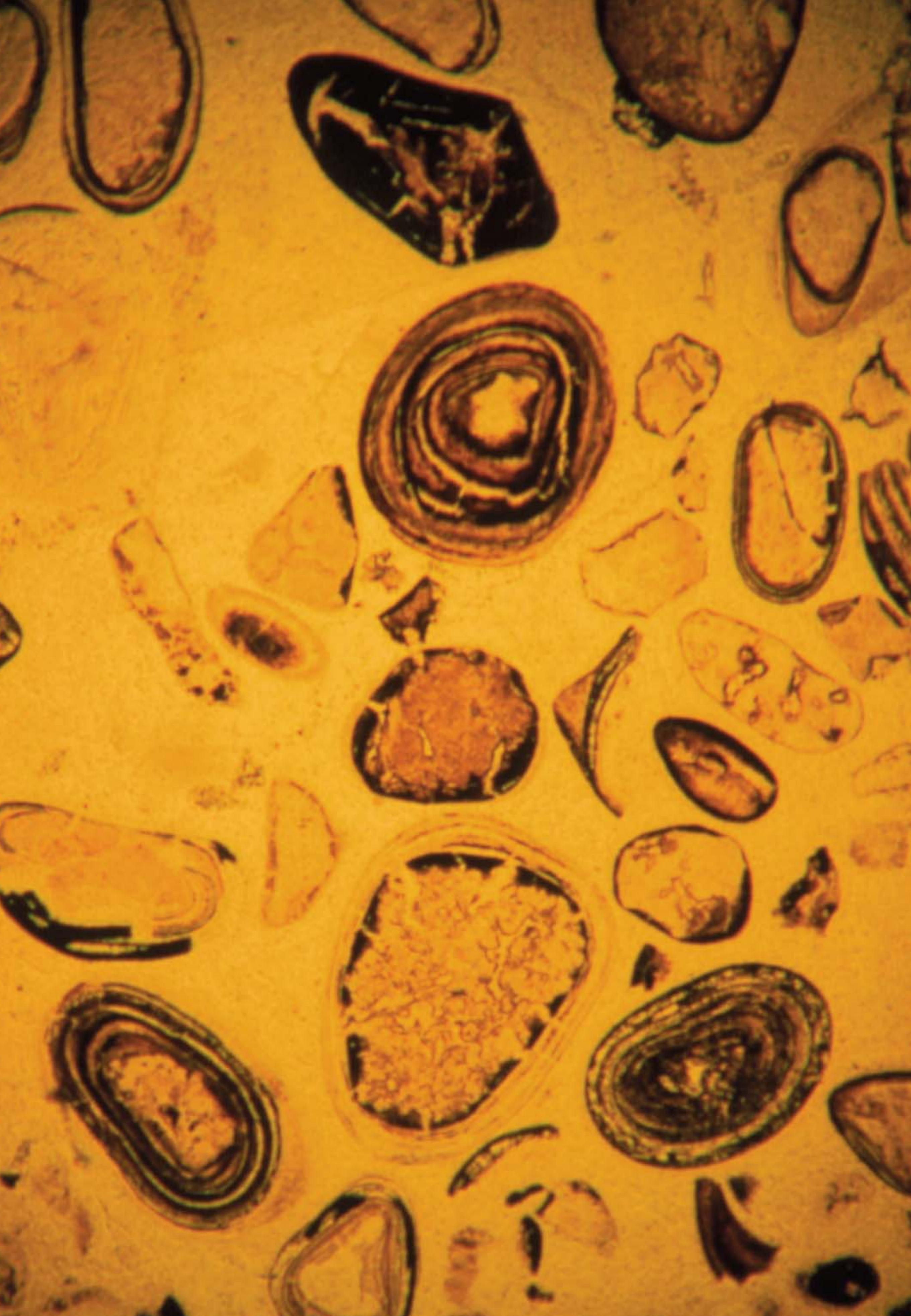

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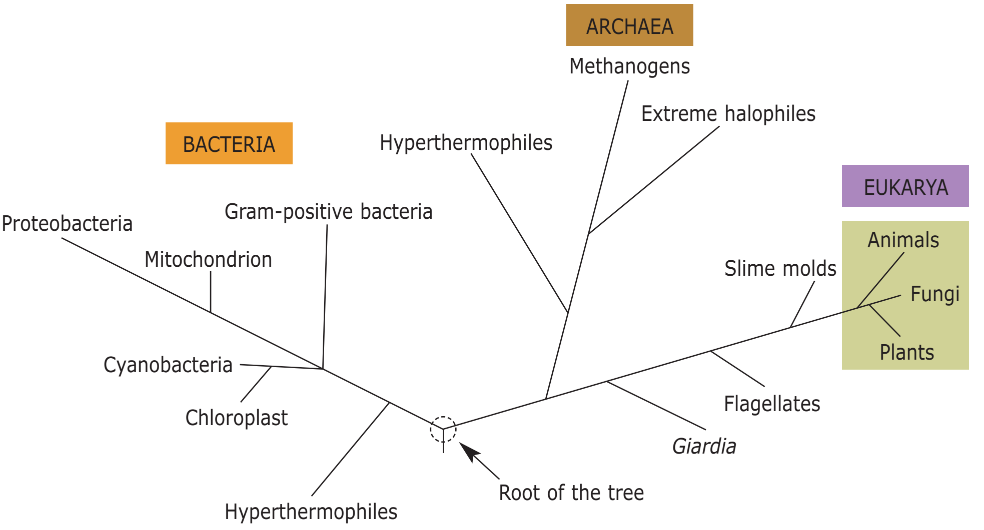
At this very instant, the light (as electromagnetic radiation in the 300-700 nm range) reflected from this written page is being absorbed by your eye, converted into electrical signals and transmitted down the optic nerve to the brain resulting in what we call vision. The sequence of biochemical reactions that generates this visual signal starts when light first strikes your retina, producing the photo-isomerization of the visual pigment protein, rhodopsin. This protein activates an enzymatic cascade that regulates the concentrations of other proteins controlling the photoreceptor potential in the rod cells of the retina. This entire sequence of events happens incredibly fast (in a pico-second or 10^{-12} second) and your brain should be able to receive the visual signal and use it, in both muscle actions and in generating thoughts. While the brains of most vertebrates send visual signals to various muscles to generate actions needed for survival, we would like to believe that we are the only species with the mental capacity for complex thought and understanding. For example, we understand

◂ **Photo 3.1: Thin rock section of Gunflint cherts (Ontario, Canada), showing fossil remains of the earliest life forms yet found.** The primitive plants of these colonies of algae derived energy through photosynthesis, releasing free oxygen as a by product. This had far reaching consequences for the subsequent history of the environment.

Figure 3.1: Phylogenetic tree of life based on comparative rRNA gene sequence data. Cyanobacteria comprise a large group of phototrophic bacteria.



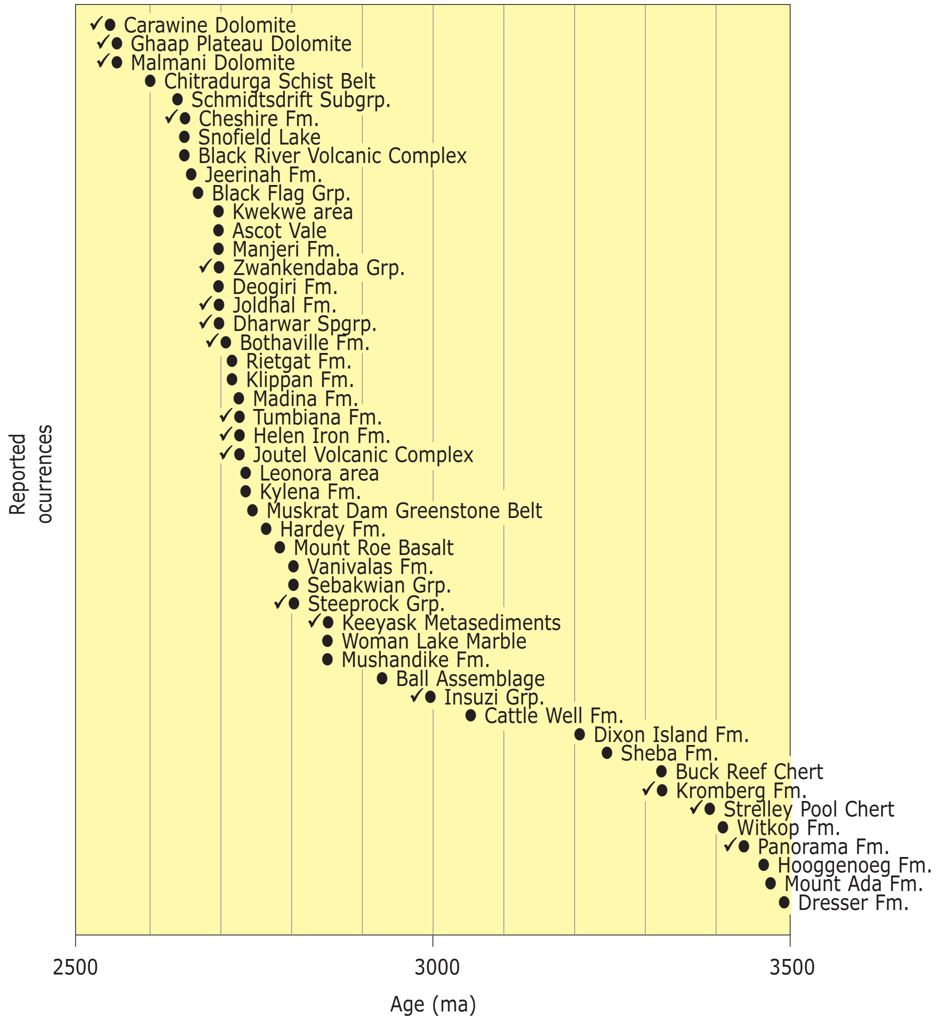
Source: Madigan et al. 2009.

that visual signals are the absorption and transduction of light into electrical signals transmitted through retinal nerve cells to the brain. As you can imagine (maybe another unique ability of the human brain), our brain is a very sophisticated organ and we are just starting to understand how our brain cells process information and generate thoughts, dreams, beliefs, consciousness, and pose questions such as how cyanobacteria made this planet habitable for humans. The main goal of this chapter is to illustrate how the metabolic activity of this group of bacteria permits our existence on this planet by producing the O_2 required for respiration in general, and enables us to maintain an energetically expensive large brain such as ours, in particular. In addition, this group of bacteria is largely responsible for producing bioavailable nitrogen required for the billions of proteins synthesized in our bodies every second to keep us alive, proteins such as the one that allowed you to read these sentences just now.

3.1. WHAT ARE CYANOBACTERIA?

Cyanobacteria comprise one of the major phyla of bacteria (figure 3.1), consisting of a large, morphologically and ecologically heterogeneous group of phototropic bacteria (Whitton and Potts 2000). Cyanobacteria are photosynthetic prokaryotes and as described below, they have transformed the chemical composition of the atmosphere and fundamentally changed the climate of planet Earth. These changes allowed the evolution of multicellular life. Geochemical, geological and paleontological evidence suggests that cyanobacteria are one of the oldest groups of bacteria on Earth. This is indicated by the abundance and widespread distribution of fossilized Proterozoic cyanobacterial communities known as stromatolites (figure 3.2). In fact, the Proterozoic Era

Figure 3.2: Archean geological units that contain fossil stromatolites. Check marks denote conical stramolites indicative of the presence of photosynthetic and phototactic microbes.



Source: Schopf 2006.

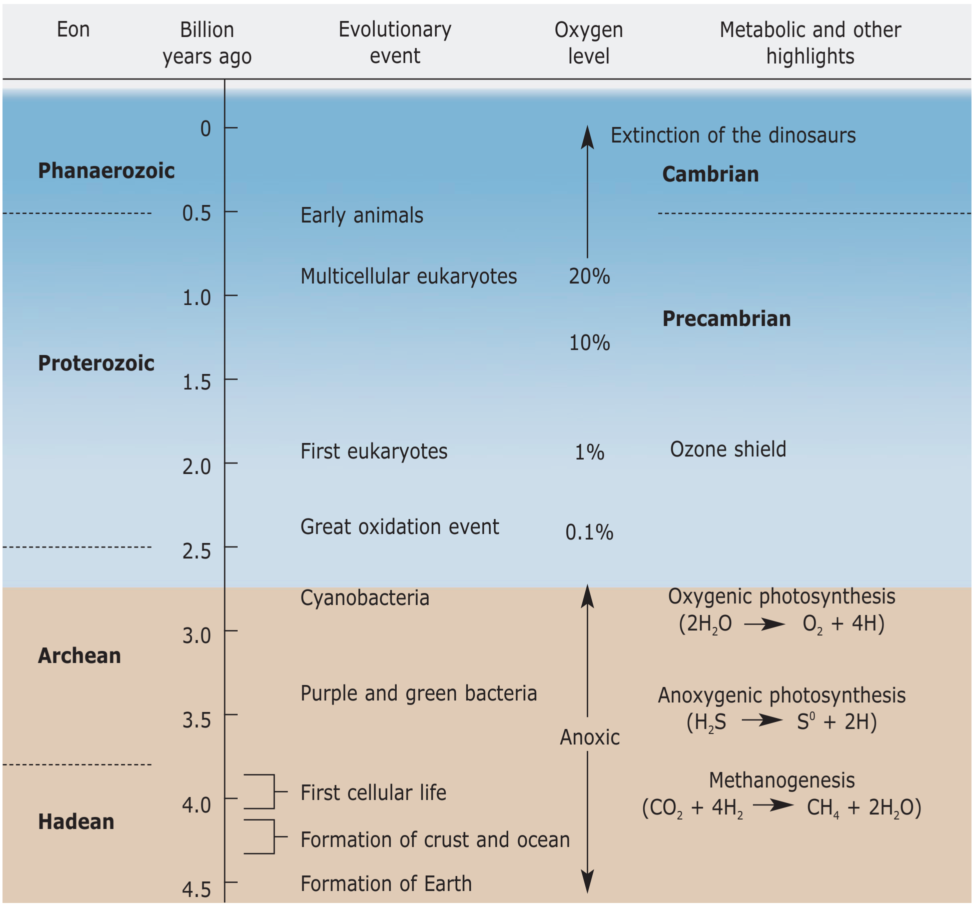
(2500-570 Ma) has been defined as the "Age of Cyanobacteria" because of their high abundance in the fossil record (Schopf and Walter 1982). The documented fossil record also shows the continuous presence of cyanobacteria back to at least 3500 Ma ago (figure 3.2). Therefore, part of the great success of cyanobacteria is their long evolutionary history, which allows them to thrive under different environmental conditions. Their high diversity and abundance is also facilitated by their small size, which favors more rapid exchange with the surrounding environment, accelerated metabolism, rapid growth rate and cellular reproduction (Whitton and Potts 2000). All of these factors have direct consequences for the adaptability and potentially high rate of evolution among the cyanobacteria. They are widely distributed in all types of habitat and in general they are more tolerant of extreme environments than are algae (Whitton and Potts 2000). Falkowski and Raven (1997) calculated that at any given time there are about 10^{25} cyanobacteria cells in the ocean. Therefore, the vast majority of photosynthetic carbon and nitrogen fixation in the contemporary ocean is carried out by this one group of living organisms.

During photosynthetic carbon fixation, cyanobacteria produce O_2 as a waste product. In fact, cyanobacteria are the only phototrophic prokaryotes capable of carrying out oxygen-producing, plant-like photosynthesis. Without that waste product, the evolution of complex multicellular organisms, including us, would not have been possible.

3.2. THE IMPORTANCE OF ATMOSPHERIC OXYGEN ON EARTH

Take a breath. If you are at this moment close to sea level, the chemical composition of the air that you are breathing is mostly N_2 (78%) and O_2 (21%). However, it was not always like that. During the first 3 billion years after the Earth's formation, the atmosphere was devoid of O_2. The great number of organisms that currently live in oxygen-free environments attests to the fact that the availability of O_2 is not a requirement for "life", but our (human) existence requires molecular oxygen. In fact, lots of oxygen. We have the largest brain of all the apes, and the energetic cost of that large brain is very high. For instance, in a human body at rest, adenosine triphosphate (ATP) molecules are formed and reformed at a rate of about 9×10^{20} molecules per second, equivalent to a turnover rate of 65 kg per day, with much higher rates during periods of strenuous activity (Rich 2003). The human brain makes up about 2% of a person's weight, but it consumes 20% of the body's energy at rest. The

Figure 3.3: Temporal sequence showing the landmarks in biological evolution, Earth's changing geochemistry and microbial metabolic diversification



Source: Madigan et al. 2009.

only element than can produce that much energy is oxygen (the typical adult male requires about 380 liters of O_2 per day, or 7600 liters of air per day).

From the time of Earth's formation, the early ocean and atmosphere were anoxic (without oxygen). Therefore, it is believed that life originated in an oxygen free-environment, and remained strictly anaerobic (generating energy without using oxygen) for more than a billion years. Then cyanobacteria evolved the ability to split water using the energy of sunlight, causing free oxygen to appear in the atmosphere for the first time (figure 3.3). In fact, all of the atmospheric oxygen found on this planet is of biological origin and started with the evolution of oxygenic photosynthesis by cyanobacteria about

2 billion years ago. The production of O_2 during photosynthesis, shown in the following overall and light reactions, seems remarkably simple. However, because water is a very stable compound, its oxidation to molecular oxygen in the light reaction is considered to be one of the major landmarks in biological evolution, transforming the life, the chemical composition and the climate of this planet forever (figure 3.3).

$$\text{Overall reaction:} \quad CO_2 + H_2O \xrightarrow{\textit{Light}} (CH_2O)n + O_2$$

$$\text{Light reaction:} \quad 2\,H_2O \xrightarrow{\textit{Light energy}} O_2 + 4\,H^+ + 4\,e^-$$

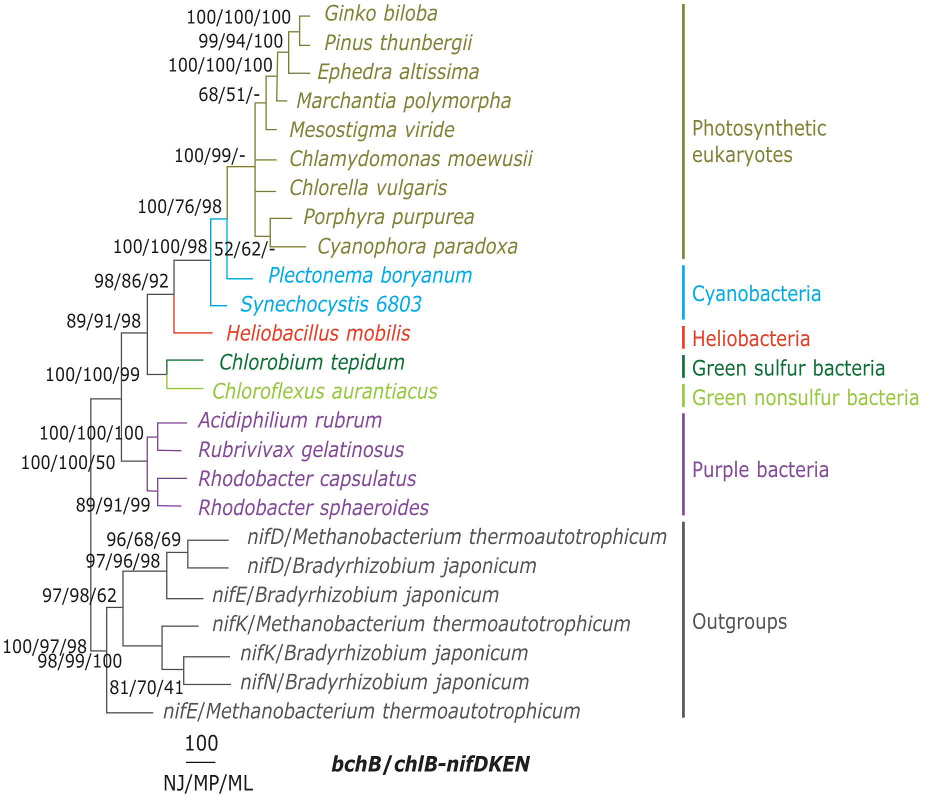
$$\text{“Dark” reaction:} \quad CO_2 + 4\,H^+ + 4\,e^- \longrightarrow (CH_2O)n + H_2O$$

3.3. ORIGIN OF PHOTOSYNTHESIS

Because the splitting of water requires a powerful oxidizing agent, major evolutionary developments were required before the oxidation of water could take place. As with any other metabolic pathway that evolved billions of years ago, it is difficult to establish who the first photosynthetic organisms were. However, relatively recent molecular evidence has identified purple bacteria as the earliest photosynthetic bacterial lineage, which later diverged into green nonsulfur bacteria, green sulfur bacteria, heliobacteria, cyanobacteria and finally to photosynthetic eukaryotes (figure 3.4; Xiong et al. 2000). However, other studies have identified heliobacteria or green nonsulfur bacteria as the earliest-evolving phototrophs (Gupta et al. 1999). Although it is still unclear in which bacterial lineage photosynthesis evolved, it is well established that oxygenic photosynthesis was a cyanobacterial invention (Xiong et al. 2000).

Photosynthesis is the biological process by which light energy is transformed into chemical energy in the form of sugar and other organic molecules. Bacterial photosynthesis carried out by purple, green nonsulfur, green sulfur and heliobacteria does not generate O_2 as a by-product. The reason is that these anoxygenic photosynthetic bacteria contain only one type of photosynthetic reaction center (Photosystem I or II), whereas cyanobacteria contain both systems and carry out both photochemical reactions in parallel (figure 3.5). Both systems are needed to use water as an electron source, as PSI or PSII alone don’t have the oxidizing power to cleave water. The oxidation of water

Figure 3.4: Photosynthesis phylogeny based on the *bch*B/*chl*B gene

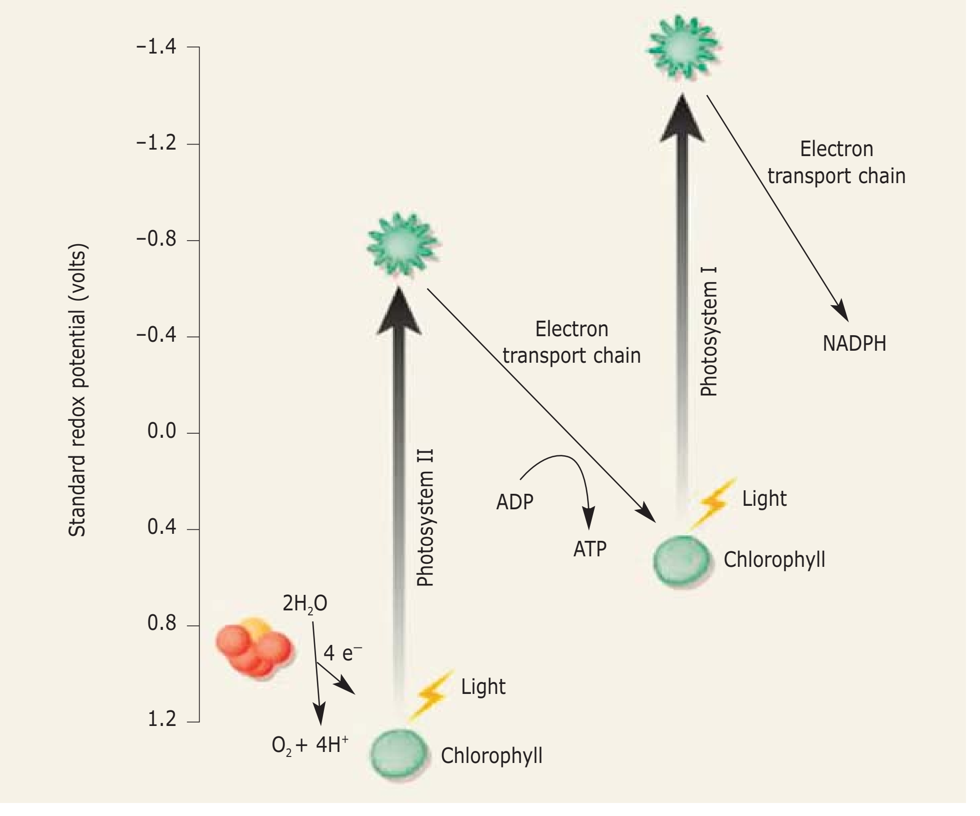


Source: Xiong et al. 2000.

was only possible after cyanobacteria evolved a reaction center pigment with a greater oxidizing potential, chlorophyll a. Anoxygenic photosynthetic bacteria contain bacteriochlorophylls as reaction center pigments. Because bacterichlorophylls absorb longer wavelength and therefore lower energy light, these reaction centers don't have the reducing power to oxidize water.

While the appearance of anoxygenic photosynthesis on Earth would have made some areas with abundant abiotic chemical sources of reducing power (e.g., S, Fe^{2+}, Mn^{2+}, H_2 and CH_4) from rock weathering and shallow hydrothermal systems more productive, the geographical distribution of organisms was still very limited due to the very localized sources of those electron donors. Because liquid water is abundant on the surface of the planet, the ability to oxidize water gave phototrophic organisms a nearly infinite source of electrons, and the ability to colonize every single environment

Figure 3.5: The two photosystems needed to split water during oxygenic photosynthesis

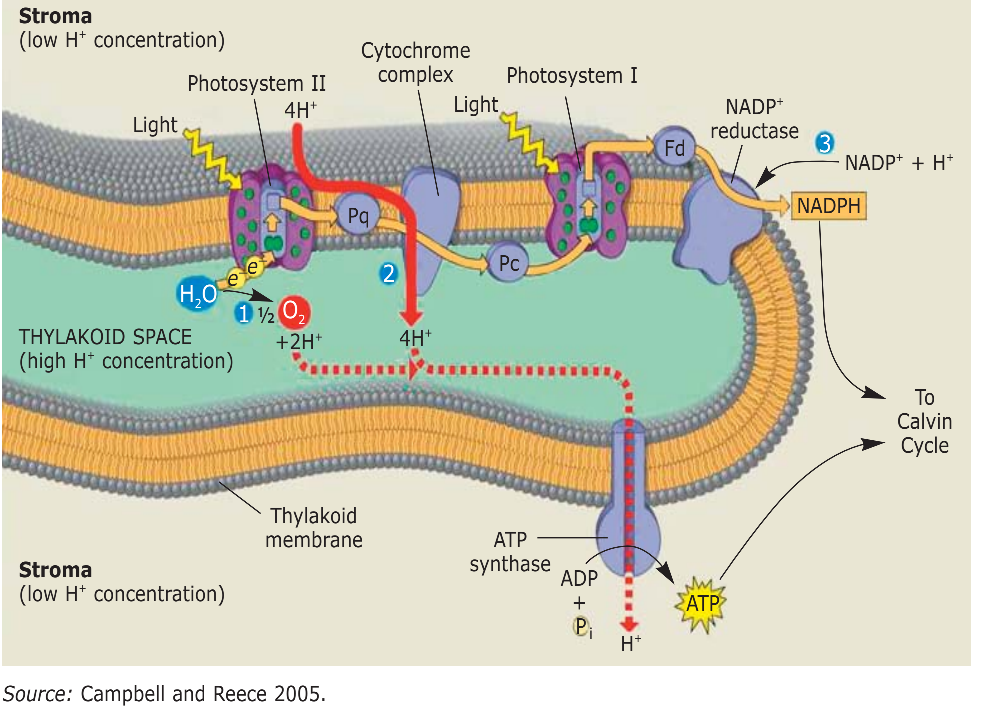


Source: Allen and Martin 2007.

on Earth. In fact, oxygenic photosynthesis underpins the existence of all life forms on the Earth's surface today.

In contrast to anoxygenic bacterial photosynthesis, oxygenic photosynthesis is a relatively recent evolutionary invention (figure 3.3). Oxygenic photosynthesis is probably the most complex energy-transducing process of life on Earth (figures 3.5 and 3.6). In order to use water as an electron-donor in oxygenic photosynthesis, organisms need a very complicated photosynthetic machinery that requires hundreds of genes, and the synthesis of a similar number of proteins clustered in two different photosystems (PSI and PSII), light-harvesting antennae, and cytochromes (Shi et al. 2005; Shi and Falkowski 2008). Furthermore, in order for the photosynthetic apparatus to work, enzymes are required for CO_2 fixation, chlorophyll synthesis and electron transport, as well as various cofactors such as pigments, hemes, Fe-S clusters and the Mn_4Ca water-splitting reaction center (figures 3.5 and 3.6). This breakthrough in bacterial metabolism transformed the chemical composition of the Precambrian period (end of the Proterozoic era) on Earth. Oxygenic photosynthesis facilitated the "great oxidation" of the atmosphere about 2.2 billion years ago, but the final oxidation of the atmosphere (to about current levels of ~20%) did not occur for another 2 billion years (figure 3.3), until tectonically driven changes caused the appearance of shelf seas where reduced organic carbon could be buried. The increased burial efficiency of organic matter on continental margins then produced an excess of global photosynthesis over global respiration, causing the rise of atmospheric O_2. This set the stage for the evolution of eukaryotic organisms (composed of cells with a nucleus and organelles) about 1.5 billion years ago. These much larger eukaryotic cells incorporated mitochondria (formerly free-living bacterial cells) as cellular organelles in which glucose is oxidized to CO_2 and water, thereby completing the energy cycle that started with oxygenic photosynthesis.

Figure 3.6: General depiction of the light reactions and chemiosmosis in the thylakoid membrane

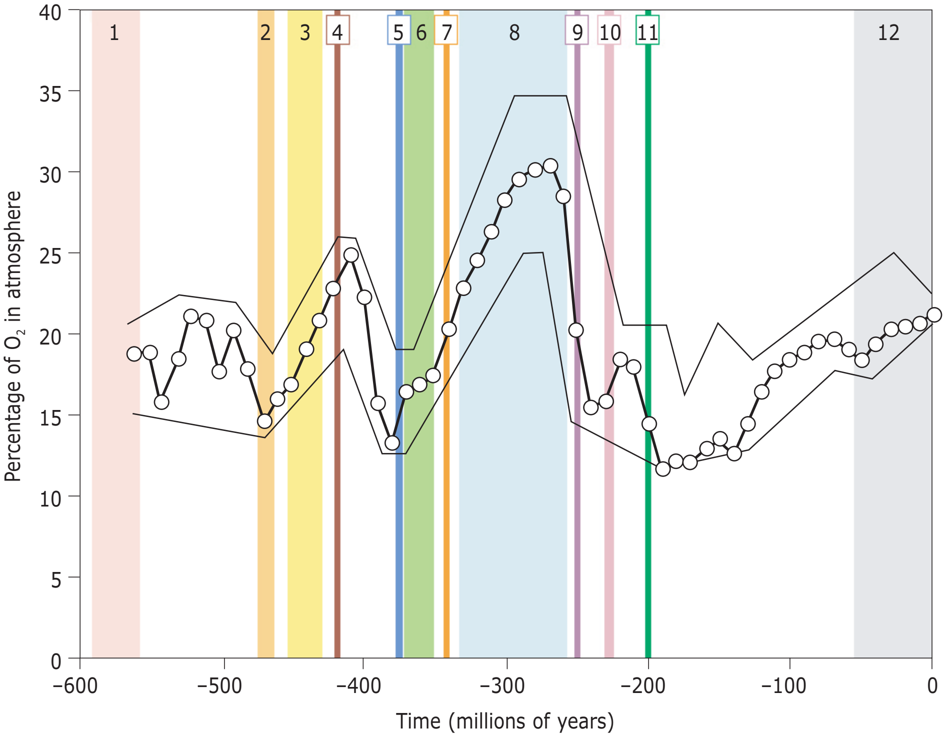


Source: Campbell and Reece 2005.

3.4. THE IMPACT OF OXYGEN ON THE DIVERSIFICATION OF LIFE

The production of O_2 during the early days of oxygenic photosynthesis caused a major ecological disaster, leading to the disappearance of most of the early anaerobic microbial life on Earth. However, as previously stated, the innovation of water oxidation opened an enormous range of new environments in which photosynthesis could occur. For the first time, life was not limited by the availability of electron donors, but only by the availability of light and nutrients. Furthermore, oxygen, the by-product of photosynthesis by cyanobacteria and their descendants (all eukaryotic photoautotrophs found in the modern world) made possible the development of more complex organisms that use more energy-efficient aerobic metabolism (Falkowski et al. 2005; Raymond and Segre 2006). For example, the evolution of metazoan (multicellular) organisms about ~0.5 billion years ago was dependent on the evo-

Figure 3.7: Potential connection between changes in atmospheric oxygen composition and major evolutionary transitions and extinction events



Source: Berner et al. 2007.

lution of oxidative phosphorylation, a very highly efficient energy recovery mechanism that requires O_2. Compared to glycolysis, this cellular respiratory process produces 18 times as much as ATP per mole of glucose, enough energy for the development of all complex multicellular organisms, including humans.

Atmospheric O_2 fluctuations seem to have played a major role as an evolutionary force on this planet, including the diversification of metazoan life forms throughout the Phanerozoic (figure 3.7). In fact, several major evolutionary transitions such as the conquest of land by animals (intervals 4 and 7 in figure 3.7), gigantism through the Carboniferous and Permian (interval 8) and the increase in mammalian body size in the Tertiary (interval 12) have all occurred under high O_2 concentrations (Berner et al. 2007). Low atmospheric O_2 concentrations seem also to have drastic consequences, as three of the major extinctions (Late Devonian (interval 5), Permian-Triassic (interval 9) and Triassic-Jurassic (interval 11: figure 3.7), all followed periods of low O_2 concentrations. One consequence of major extinctions is the rapid evolution of new metabolic pathways, including a more efficient respiratory system that requires less oxygen.

3.5. CYANOBACTERIA AND NITROGEN FIXATION

The extensive anaerobic microbial world attests to the fact that oxygen is not a required element for *all* forms of life. In contrast, all organisms (anaerobic or aerobic) require nitrogen to synthesize the proteins that make life possible. In fact, although genes get a lot of attention, it's the proteins that perform most life functions and make up the majority of cellular structures. Although there is a large nitrogen pool in the atmosphere of this planet (in fact, it is the major component in the Earth's atmosphere; about four-fifths of the air we breathe is N_2), this nitrogen is biologically unavailable because of the triple bond between nitrogen atoms (one of the strongest chemical bonds known) that makes N_2 extremely unreactive. Therefore, before N_2 can be incorporated into different bio-molecules, it has to be chemically reduced to a biologically available form of nitrogen (also called fixed nitrogen), namely, ammonia (NH_3). The critical task of fixing nitrogen for all living things is performed by a group of microorganisms, called diazotrophic prokaryotes that possess the multimeric enzyme complex, nitrogenase. In fact, all known nitrogen-fixing organisms are prokaryotes. During nitrogen fixation, nitrogenase catalyzes the reduction of molecular nitrogen to ammonia according to the following equation:

$$N_2 + 8\ H^+ + 8\ e^- + 16\ ATP \longrightarrow 2\ NH_3 + 16\ ADP + 16\ P_i + H_2$$

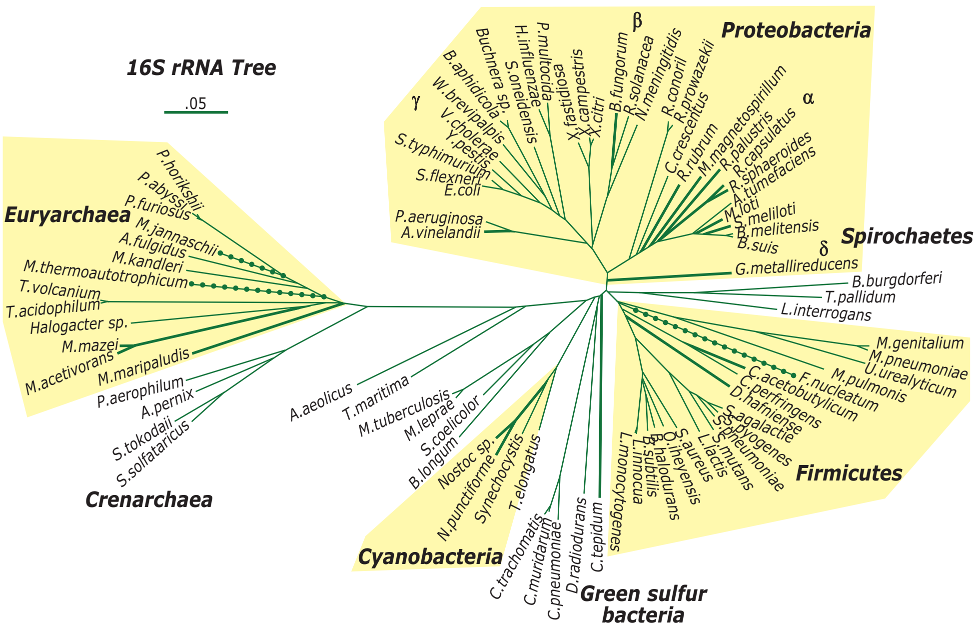
where ATP, ADP and P_i represent adenosine triphosphate, adenosine diphosphate and inorganic phosphorus respectively.

Because the triple-bond in the molecular N_2 is very stable (bond energy of 942 kJ/mol), nitrogen fixation requires an extremely high activation energy. With 16 ATPs needed to hydrolyze every mol N_2 fixed, the nitrogenase enzyme system carries out one of the most metabolically expensive processes in biology (Simpson and Burris 1984). While biological nitrogen fixation occurs at about 0.8 atm of nitrogen, the industrial synthesis of ammonia for fertilizers by the Haber-Bosch process is carried out at temperatures of 400 to 500 °C, and atmospheric pressures of N_2 and H_2 of several hundred, to obtain the necessary activation energy (Lehninger et al. 1993).

In contrast to oxygenic photosynthesis, it is difficult to establish when nitrogen fixation evolved. However, because nitrogen is necessary for the origin of life, nitrogen fixation may be a very ancient metabolic pathway. It is likely that before the evolution of biological nitrogen fixation, the only source of biologically available nitrogen was abiotic reactions produced by lightning discharges (Navarro-González et al. 2001). However, it has been hypothesized that abiotic sources of fixed nitrogen in the early Earth were limited (Raven and Yin 1998; Kasting and Sieferet 2001; Navarro-González et al. 2001), and that at some point in time (probably during the early Archaean), a reduction in the rate of abiotic nitrogen fixation (Navarro-González et al. 2001) could not support the nitrogen requirements of an expanding microbial biomass. This nitrogen crisis probably produced the evolutionary pressure behind the evolution of biological nitrogen fixation (Towe 2002). This scenario suggests that biological nitrogen fixation occurred early in the evolution of life on this planet, as diazotrophic organisms are found exclusively among prokaryotes, although they occur in both Bacteria and Archaea domains, which are not closely related (figure 3.8).

The evolutionary history of nitrogenases also suggests that biological nitrogen fixation is an ancient process. In an anoxic atmosphere containing N_2 and CH_4, photochemical reactions could have produced large amounts of triple-

Figure 3.8: Prokaryotic phylogenetic tree. Diazotrophs are indicated with bold green lines.



Source: Raymond et al. 2004.

bonded compounds such as C_2H_2 (acetylene) and HCN (hydrogen cyanide) (Kasting and Siefert 2001). Therefore, it is possible that this ancestral enzyme acted as a detoxifying agent, used by organisms for protection against acetylene, cyanide, azide, or any other N_2 analog formed in early anoxic atmosphere (Silver and Postgate 1973).

Because fixed nitrogen was necessary for synthesis of the biomolecules that sustain life on this planet, it is not surprising that the ability to fix nitrogen is found in both aerobic and anaerobic environments, and throughout the prokaryotic world, including in the cyanobacteria (figure 3.8), the same bacterial group that "invented" oxygenic photosynthesis. However, although nitrogen fixation genes have only been found in three of the six classified orders of cyanobacteria (Raymond et al. 2004), diazotrophic cyanobacteria contribute substantial amounts of fixed nitrogen in marine environments. This is relevant because most of the world's oceans are depleted in inorganic nitrogen at the surface, and therefore, in those expansive open ocean environments, the net biological activity depends on the amount of fixed nitrogen available. Current estimates of global N_2 fixation are ~240 Tg N yr^{-1} with a marine contribution of

100-190 Tg N y^{-1} that is between 40 to 80% of the global N_2 fixation (Berman-Frank et al. 2003). The critical role played by cyanobacteria in the marine nitrogen cycle is demonstrated by the fact that all marine nitrogen fixation is carried out by these organisms. Therefore, without the N_2 fixation carried out by the small cyanobacteria, most of the world's oceans would be devoid of life.

In summary, in a time when climate change, environmental degradation, overpopulation, conspicuous consumption and another long list of maladies jeopardizes the long-term survival of human societies, I have tried to provide in this chapter a sense of how tiny cyanobacteria have transformed the chemical environment of this planet and eventually allowed the evolution of humans. Because the splitting of water during oxygenic photosynthesis and the destruction of the nitrogen triple-bond during nitrogen fixation are not thermodynamically favored, our life on the surface of this planet, based on solar energy, may be an exception rather than the rule. I hope this chapter will help the reader to appreciate our planet in a different way and to see that luck has been on our side. How long this luck will last is up to us.

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