Geomicrobiology: Molecular and Environmental Perspective
Larry L. Barton • Martin Mandl
Alexander Loy
Editors

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Preface

The interaction of microorganisms with geological activities results in processes influencing development of the Earth’s geo- and biospheres. In assessing these microbial functions, scientists have explored short- and longterm geological changes attributed to microorganisms and developed new approaches to evaluate the physiology of microbes including microbial interaction with the geological environment. As the field of geomicrobiology developed, it has become highly interdisciplinary and this book provides a review of the recent developments in a cross section of topics including origin of life, microbial-mineral interactions and microbial processes functioning in marine as well as terrestrial environments. A major component of this book addresses molecular techniques to evaluate microbial evolution and assess relationships of microbes in complex, natural communities. Recent developments in so-called ‘omics’ technologies, including (meta)genomics and (meta)proteomics, and isotope labeling methods allow new insights into the function of microbial community members and their possible geological impact. While this book summarizes current knowledge in various areas, it also reveals unresolved questions that require future investigations. Information in these chapters enhances our fundamental knowledge of geomicrobiology that contributes to the exploitation of microbial functions in mineral and environmental biotechnology applications. It is our hope that this book will stimulate interest in the general field of geomicrobiology and encourage others to explore microbial processes as applied to the Earth. Not only have authors provided skillful reviews but also they have outlined unique perspectives on environmental microorganisms and their related processes. This book should be of interest to scientists of various fields including biogeochemistry, geology, microbiology, microbial ecology, evolution, and environmental sciences.

We appreciate the efforts of the authors who have generously contributed their time to prepare chapters for this book and we encourage readers to consult them for further advancements in their research areas.

Larry L. Barton
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Chapter 1
Chemoautotrophic Origin of Life: The Iron–Sulfur World Hypothesis

Günter Wächtershäuser

Introduction

The study of the origin of life is an immature science. If we apply the strictures of Immanuel Kant it may not be considered a mature science until it can be said to have embarked on a course of orderly progress. Indeed, if we review the development of research into the origin of life, we have to admit that it is still far from presenting the image of progress. It may be best characterized as an exercise of randomly groping around – and doing so at a number of different levels.

At the philosophical level we still are faced with the conflict between mechanistic explanations and teleological judgments. Biochemistry is providing ever more refined mechanistic explanations of the chemistry of life, down to the finest molecular details. A theory of biology, by contrast, would have to treat organisms, i.e. organized beings, as integrated wholes. Biochemistry is reductionistic and mechanistic while biology is holistic and teleological. This provides us with our first question. What would count as a solution of the problem of the origin of life – a molecular reaction mechanism or a primordial organism? Kant of course held that a natural science would be impossible without mechanistic explanations (Kant 1790). Applying this requirement he suggested that a full replacement of all teleological notions of “natural purposes” (end-means relations or functions within a whole organism or between an organism and its environment) by mechanisms would be impossible. Kant did see a “ray of hope” that a mechanistic theory of evolution, i.e. of transformations from one type of organism to another might one day be achievable. He was, however, convinced that a scientific theory of the origin of life, defined as replacement of all teleological judgments by mechanistic explanations, would be impossible. It would require postulating an ultimate ancestral organism, which would have to be endowed with the
means toward the ends of all future organisms, and so it would remain inescapably teleological. This then is our problem: to postulate a primordial organism – here termed “pioneer organism”, which is at the same time mechanistic and organizational. Central to this effort will be the notion of a “synthetic autocatalysis”, the chemical equivalent of biological reproduction, which is a chemical reaction mechanism and at the same time a functional whole, and by being synthetic it is endowed from the start with the primary vector of complexity increase.

The problem of the origin of life is situated within three major scientific disciplines: biology, geology and chemistry. Biology and geology are both sciences of natural history, interconnected in a multitude of ways. Geological processes provide the habitats of life and products of organismic activities leave their mark in the compositions of the atmosphere, the hydrosphere and the lithosphere of the Earth; and in the bio-geo-chemical cycles: the carbon cycle, the nitrogen cycle and the sulfur cycle. In these cycles geochemical and biochemical reaction segments are integrated. Therefore, broadly speaking, the problem of the origin of life is the problem of the origin and early evolution of the bio-geo-chemical connection.

In principle we may think of two different possible sources of evidence for the origin and early evolution of life: geological evidence locked in the rocks of the extant lithosphere and biochemical evidence conserved in the extant biosphere. The global process of plate tectonics seems to have obliterated any rock formation old enough for bearing witness to the origin and earliest evolution of life on Earth. This leaves us with the second source of evidence, the biochemical record. The most astounding fact of this record is its complexity in terms of both molecular structures and reaction networks. In view of this complexity the best we could hope for would be an initial theory that points in the right general direction and a program of theory evolution towards ever increasing organismic and evolutionary comprehensiveness and ever finer molecular detail. For establishing such an initial theory we need a heuristic that correlates known facts of extant biochemistry with the unknown chemistry of the pioneer organism.

The conventional heuristic of “backward projection” establishes one-to-one relations between extant biochemical features and chemical features of the origin of life (Fig. 1.1a). The repeated application of this heuristic leads to a recipe for life’s beginning that comprises a large number of compounds each identical with or similar to an extant bio-compound. These are all projected into an aqueous concoction, widely known by the names “prebiotic broth” or “primordial soup”. Its birthmark is obscurity. Nobody has ever been able to specify a chemically convincing mechanism, by which the first organism could have appeared in a prebiotic broth.

By contrast, the heuristic of “biochemical retrodiction” (Wächtershäuser 1997) generates many-to-one relationships, whereby several different extant biochemical features are correlated to one simpler functional precursor feature. By employing this heuristic to include more and more extant features we move to ever deeper, fewer and simpler precursor features. In this manner the heuristic of biochemical retrodiction generates an overall pattern of backward convergence (Fig. 1.1b). It is immediately apparent that this heuristic does not lead into the chaos of a prebiotic broth. It rather focuses on one specific, coherent chemical entity, the “pioneer organism” of life.
When we view these correlations not in the backward heuristic direction, but in the forward evolutionary direction, we speak of an explanatory connection between the hypothetical pioneer organism and extant biochemistry. In any event, the heuristic of biochemical retrodiction remains methodological and its results remain problematic and in need of empirical evaluation and improvement. Chemical testing provides the best means for an empirical evaluation. In spite of its historic nature a theory of the origin of life may readily satisfy the logic of empirical testing. For example, if a theory of the origin of life requires a certain chemical reaction and if we accept experiments that show this reaction to be impossible, we are forced to admit the falsity of that theory of the origin of life. Theories on the origin of life, therefore, have the same logical status as all other scientific theories.

At the very origin of life there must be a phase transition between the pioneer organism and its aqueous environment. Recent findings point to the availability of liquid water on Earth as early as 4.4 billion years ago and the origin of life may date back to this time (Wilde et al. 2001; Peck et al. 2001; Mojzsis et al. 2001). Most notions of geological uniformitarianism break down when we enter this early hadean period of Earth’s history. Therefore, all geochemical clues concerning the parameters of the origin of life are highly problematic. Moreover, the pioneer organism must be seen as a coherent entity in a spatially and temporally coherent situation. This requirement of coherence puts severe constraints not only on the possible sets of reaction parameters, but also on the possible sites of habitats of the pioneer organism.

**Retrodicting the Origin from the Chemical Elements of Life**

We are long used to it by now, but it should still fill us with a sense of wonder that by the universal laws of nature and by a few natural constants a total of 80 stable elements are enabled, each a discrete chemical individual with discrete chemical properties.
All these chemical individuals are organized in the Periodic System of elements. When we rank the elements in the order of decreasing abundance in our Solar System (Lodders 2003), we note that the most abundant elements of the Solar System comprise also the most important elements of life as shown below (bold letters indicating the bio-elements; numerals in parentheses indicating the abundance as numbers of atoms relative to an arbitrary numerical value of $10^6$ for the abundance of Si):

$$\text{H}(2.43 \times 10^{10}) > \text{He} > \text{O}(1.413 \times 10^7) > \text{C}(7.079 \times 10^6) > \text{Ne} > \text{N}(1.95 \times 10^6) > \text{Mg}(1.02 \times 10^6) > \text{Si}(\equiv 10^6) > \text{Fe}(8.38 \times 10^5) > \text{S}(4.449 \times 10^5) > \text{Al} > \text{Ca}(6.287 \times 10^4) > \text{Na} > \text{Ni}(4.93 \times 10^4) > \text{Cr} > \text{Mn}(9.168) > \text{P}(8.373) > \text{Cl} > \text{K} > \text{Ti} > \text{Co}(2.323) > \text{Zn}(1.226) > \text{F} > \text{Cu}(527) > \text{V}(288.4) > \text{Ge} > \text{Se}(65.79) > \text{Li} > \text{Kr} > \text{Ga} > \text{Sc} > \text{Sr} > \text{Zr} > \text{Br} > \text{Rb} > \text{As} > \text{Te} > \text{Y} > \text{Xe} > \text{Ba} > \text{Sn} > \text{Pb} > \text{Mo}(2.601) > \text{Ru} > \text{Cd} > \text{Pd} > \text{Pt} > \text{Ce} > \text{I} > \text{Nd} > \text{Nb} > \text{Be} > \text{Os} > \text{Ir} > \text{Ag} > \text{La} > \text{Hg} > \text{Dy} > \text{Rh} > \text{Cs} > \text{Gd} > \text{Sb} > \text{Er} > \text{Sm} > \text{Yb} > \text{Au} > \text{Th} > \text{In} > \text{Pr} > \text{Hf} > \text{Bi} > \text{W}(0.1277) > \text{Eu} > \text{Ho} > \text{Th} > \text{Re} > \text{Tm} > \text{Lu} > \text{Ta}.$$

The 20 bio-elements fall into three chemically distinct clusters in the periodic system. The seven main group non-metal bio-elements hydrogen (H), carbon (C), nitrogen (N), oxygen (O), phosphorus (P), sulfur (S) and selenium (Se) form inorganic, small molecule compounds that are gaseous or volatile and escape from the interior of the Earth as volcanic exhalations, notably as dihydrogen ($H_2$), methane ($CH_4$), dinitrogen ($N_2$), hydrogen sulfide ($H_2S$), hydrogen selenide ($H_2Se$), carbon monoxide ($CO$), carbon dioxide ($CO_2$), carbon oxysulfide ($COS$), carbon oxyselenide ($COSe$), hydrogen cyanide ($HCN$), methyl mercaptan ($CH_3SH$), sulfur dioxide ($SO_2$), selenium dioxide ($SeO_2$), phosphorus pentoxide ($P_4O_{10}$). These small molecules constitute the ultimate nutrients for the metabolism that forms the bulk organic compounds of all extant organisms. Steam ($H_2O$) constitutes the dominant compound in volcanic gases and it condenses to liquid water, which is the universal medium of life. Its properties are uniquely suited for transporting nutrients, facilitating redox and acid–base reactions, and providing the medium for the phase separations that form the basis of the organism–environment dichotomy of life.

The nine transition metal bio-elements vanadium (V), molybdenum (Mo), tungsten (W), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu) and zinc (Zn) are located in minerals of the crust of the Earth. Their function in biochemistry is mainly catalytic owing to their abilities to cycle through different redox states and to acquire diverse ligand coordination geometries. They form the catalyst centers of metallo-enzymes. The four main group metal bio-elements sodium (Na), magnesium (Mg), potassium (K), calcium (Ca) are also located in minerals in the crust of the Earth. The alkali metals Na and K are subject to facile leaching as mono-cations from these minerals. The alkaline earth metals Mg, Ca are di-cationic and remain essentially fixed in minerals. They operate mainly as counter ions and as structure-forming bridges for anionic groups in the organic constituents of life. The absence of abundant chromium (Cr) from the set of bio-metals may be explained by its tendency to form weak bonds with sulfur, which is not true for any one of the transition metal bio-elements. The presence of the ultra-rare transition metal tungsten (W) in the set of bio-metals may be explained by its increased abundance in volcanic/hydrothermal settings and the presence of molybdenum (Mo) may be due to a relatively late replacement of tungsten (W).
The distinction between main group non-metal bio-elements and transition metal bio-elements constitutes perhaps the deepest functional dichotomy in life. Carbon and the other main group non-metal elements are pre-ordained to form the required range of molecular structures owing to the ability of carbon to form the C–C bonds in diverse molecular skeletons, that are stabilized by having their valences occupied by hydrogen. Moreover, the ability of carbon to form relatively stable bonds to O, N, S, Se is responsible for the formation of the functional groups of the bioorganic compounds that provide reactivity and form the basis for their ability to aggregate to larger structures. The transition metal bio-elements form lumped coordination structures with a transition metal center that is surrounded by a ligand sphere. They are pre-ordained to function as catalysts, notably in the fixation of volcanic gases. Thus, the main group non-metal bio-elements are mainly structural, while the transition metal bio-elements are mainly catalytic.

The fundamental dichotomy of the bio-elements has been overlooked for the longest time. This is partly due to the misconception that the most abundant bulk material in extant cells must also be the most ancient material of life. Already Carl von Nägeli in his groundbreaking theory on the origin of life assumed that the bulk material of extant organisms, i.e. proteins, constituted the original material of life (Nägeli 1884). His proposal resurfaced 40 years later, when a young Russian student, A. I. Oparin, after returning from his studies in Kossel’s laboratory in Heidelberg, Germany, published a theory on the origin of life (Oparin 1924) and variants thereof continue to be proposed to this day. After it became clear in the 1940s that the nucleic acids are the molecules of inheritance in extant organisms, it was suggested that life began with replicating nucleic acids (in a prebiotic broth), known today as “RNA-world” theories (Woese 1967; Orgel 1968). All these conventional theories associate the origin of life with the onset of replicative polycondensation of low molecular weight monomers to high molecular weight polymers or aggregates thereof. The chemical synthesis of the monomers is relegated to an obscure chemical evolution in the “prebiotic broth”, wherein the monomers are supposed to have accumulated and become activated over thousands or millions of years. All “prebiotic broth” theories suffer from the same paradox: they have to postulate liquid water as the universal medium of life and of the prebiotic broth. Yet it is precisely this medium that tends to counteract the formation and accumulation of polycondensation products (activated monomers or polycondensation agents). The RNA world theories have the added problem of an intramolecular catalysis of RNA hydrolysis due to the 2′-OH groups of the ribose rings. This aggravates the hydrolysis problem considerably, notably at elevated temperatures and under alkaline conditions. The assumption of RNA molecules as carriers of genetic information, notably over longer geologic periods of time, is therefore chemically unreasonable. Contrary to common perceptions there is not a single example for an RNA genome under cellular conditions. In RNA viruses the RNA genome is packaged under stabilizing neutral or even water-free conditions within an impervious casing. As soon as the viral RNA genome enters a host cell is rapidly replicate or its information is incorporated into the host DNA genome. For these reasons all the popular RNA-world theories are chemically unreasonable.
and symbolic for the distance that exists between origin-of-life research and level-headed chemical knowledge.

The above difficulties seem to be insurmountable and render any “prebiotic broth” theory at the level of the formation of polymers from monomers a lost cause. Wouldn’t it then be about time to seriously consider a radically new approach: To see whether it would not be possible to locate the onset of the pioneer organism at the level of the formation of low molecular-weight organic compounds from small inorganic molecules as they are found in volcanic exhalations. Such a theory has been proposed in 1988 for a volcanic flow setting, and it has evolved over the subsequent years (Wächtershäuser 1988a, b, 1990, 1992, 1997, 1998a, 2001, 2003, 2006, 2007) toward greater detail and greater comprehensiveness, which now goes up to the origin of the domains Bacteria, Archaea and Eukarya. The theory departs from conventional notions on several points:

1. The origin of life is associated with the synthesis of low-molecular weight organic compounds from inorganic nutrients.
2. The origin of life is identified with a seemingly instantaneous induction of synthetic, autocatalytic carbon fixation (termed “chemoautotrophic origin”).
3. The origin of life is dependent on redox reactions, for which the presence of liquid water is a benefit rather than a detriment. Redox reactions are chemically “creative” with thoroughgoing changes of the electron configurations, while polycondensation reactions are merely organizational, leaving the electron configurations of the monomers essentially unchanged.
4. The origin of life is critically dependent on transition metal catalysis.
5. The origin of life is associated with a dynamic inheritance of “analogue” information in the form of autocatalytic feedback effects while static inheritance of “digital” sequence information is seen as the result of evolution.
6. The origin of life is associated with the redox potential of kinetically inhibited, quenched (non-equilibrium) volcanic exhalations.

On the Minimal Organization of the Pioneer Organism

The notion of a pioneer organism based on the onset of autocatalytic carbon fixation requires a sufficient compositional coherence for preventing a rapid decrease of product activity by diffusion, while permitting uninhibited access of nutrients. This requirement leads us to postulate a minimal organization in the form of a composite structure (Fig. 1.2). It consists of an organic superstructure attached to an inorganic substructure, which may be amorphous or crystalline; compact, porous or layered; aggregated or agglomerated; suspended or packed.

The superstructure consists of low-molecular weight organic compounds, which are formed by reductive autocatalytic carbon fixation pathways that are catalyzed by the transition metal centres in or on the inorganic substructure. The organic compounds come to be bonded in statu nascendi as ligands onto the transition metal centers, whereafter they engage in a surface metabolism. This minimally organized
entity with the totality of the organic molecules that are bonded at any given time to outer (or inner) surfaces of the inorganic substructure (plus the proximal regions of the inorganic substructure) is defined as the pioneer organism. The pioneer organism is indefinite in terms of the profiles and lateral extensions of the surfaces of the substructure. In the direction normal to the outer or inner surfaces the organic superstructure has a definite extension and a vectorial orientation. The water phase constitutes the environment and the source of nutrients.

The so-called iron group elements Fe, Co, Ni are the most effective and versatile catalysts of life. Among these Fe is most outstanding and also of greatest geochemical abundance. Moreover, the most stable compounds of the iron group elements and notable of Fe under anaerobic conditions are the sulfides, while in a metabolic context the most prominent forms are clusters with sulfur ligands. Therefore, the theory of a chemoautotrophic pioneer organism under volcanic conditions has been dubbed “iron-sulfur world theory”.

The organic superstructure is a dynamic entity. It has a steady state composition determined by the rates of synthesis and by the rates of detachment from the substructure and subsequent irreversible dissolution in the vast expanses of the ocean. The inorganic substructure should also be viewed as undergoing dynamic changes in accordance with the “Ostwald-Volmer step rule”: The withdrawal of energy from a chemical system that can exist in several states of density will not go directly, but rather stepwise, into the state of greatest density, which is typically, but not always, also the state of greatest thermodynamic stability. In this sense the initial substructure may be a highly energetic, amorphous hydrated structure. It subsequently becomes denser and stabilizes by dehydration and crystallization. In fact, the initial substructure may be interpreted as an extended polynuclear, polymodal, heteroleptic complex. Its ligands may at first be mainly hydroxy and aquo ligands.

Fig. 1.2 Minimum organization of the pioneer organism
The later development is characterized by a competitive input of polymerizing bidentate ligands (sulfidation or cyanidation) and of depolymerising ligands (carbonylation or ammination). Sufficient sulfide activity will ultimately drive this stabilization cascade to the formation of transition metal sulfide crystals. Notably iron, the most abundant of all transition metals may form at first ferrous hydroxide, which undergoes sulfidation to ferrous sulfide as poorly ordered crystals. These will subsequently go down a cascade of stabilizations with oxidative pyrite formation as a source of reducing power at the end stage,

$$\text{FeS} + \text{H}_2\text{S} \rightarrow \text{FeS}_2 + 2\text{H}^+ + 2\text{e}^-.$$  (1.1)

Catalytic minerals have been invoked practically from day 1 in conjunction with a prebiotic broth. Nägeli suggested for the formation of reproducing protein micells (Nägeli 1884): “Probably it does not occur in a free body of water, but rather in the wetted surface layer of a fine porous substance (clay, sand), where the molecular forces of the solid, liquid and gaseous bodies cooperate” (translation by the author). Bernal reinvented this idea later (Bernal 1951) and clay minerals have been invoked extensively ever since, mainly for catalyzing polycondensation reactions. Subsequently, Cairns-Smith (1982) took the bold step of replacing one-dimensional nucleic acid templating by two-dimensional layered clay templating, thus postulating inorganic clay organism. Arrhenius introduced divalent-trivalent metal hydroxide minerals, notably of iron, with a layered structure for the absorptive concentration and selective synthetic conversion of organic compounds in the prebiotic broth (Kuma et al. 1989). Volcanic sites were invoked early on, but merely as a source for the prebiotic broth (Mukhin 1974). Hydrothermal vents began to be suggested as sites for the origin of life soon after their discovery (Corliss et al. 1981), but without recognition of the need for volcanic quenching. Several authors engaged in a formal analysis of an origin by autocatalysis of small organic molecules, but still remained victim to the notion of a heterotrophic origin in a “prebiotic broth” (Ycas 1955; King 1977).

**Metabolic Reproduction and Evolution of the Pioneer Organism**

The most characteristic feature of the iron–sulfur world theory resides in the need for a mechanism of metabolic reproduction and evolution, by which the products of the synthetic reactions may exhibit autocatalytic feedback into the synthetic reactions themselves. Extant metabolisms comprise two aspects that must have evolved along two distinctive tracks: (1) Evolution of catalysts beginning with inorganic transition metal centers and proceeding through ligand variation to metallo-peptides and later to metallo-enzymes; (2) Evolution of biochemical pathways to more and more extended and integrated pathway systems. These two tracks are here assumed to have always been interconnected in the following manner (Fig. 1.3). A transition metal center (Me₁) catalyzes a pathway for the synthesis of
organic compounds from volcanic gases, e.g. CO, NH$_3$, HCN, H$_2$S. Some products of this pathway become in turn ligands to generate a modified metallo-catalyst. Now, it is well established that certain ligands bonded to catalytic transition metal centers may increase their catalytic activity by factors of more than $10^3$ in an idiosyncratic, theoretically unpredictable manner (Berrisford et al. 1995). Therefore, some of the ligands ($L_1$) are expected to promote the catalytic activity of the transition metal centers. The new metallo-catalyst (Me-$L_1$) augments the concentration of ligand $L_1$ (autocatalysis). This is a positive feedback effect, which in biological parlance is termed reproduction. Incidentally, from this point of view racemates of the organic product ligands mean a higher number of potential ligand structures and thus a higher likelihood of a positive feedback. This means that at the early stage of life a lack of enantioselectivity of the synthetic reactions is a benefit rather than a detriment.

Now, we have to recognize that under primordial conditions of low catalytic specificity the positive feedback of a certain ligand ($L_1$) will promote not only the formation of that ligand, but also the formation of a set of other reaction products. This means that the steady state concentrations of some other reaction products ($L_2$ … $L_n$) come up to levels, at which further feedback effects are induced. In this manner a single positive feedback effect will usher in further feedback effects. These in turn elicit new metallo-catalysts, new metabolic expansions, new ligands, and in turn new metallo-catalysts and so forth. In this manner the metabolism of the pioneer organism is subject to an avalanche of self-expansions.

Of all the products of the metabolism some will bond to transition metal centers with more or less long residence times (or retention times under flow conditions) and others may not essentially bond at all. The longer the residence time or retention time of a product on the surface the higher is the likelihood that the product is protected from destruction by hydrolysis or other reactions. In this sense the products of the pioneer metabolism are self-selective.

In addition to the metabolic self-expansion effect, we also see an expansion of the range of catalytic metals (e.g. Me$_1$ $\Rightarrow$ Me$_i$ to Me$_n$) due to ligands from the expanded set of reaction products ($L_1$ to $L_n$), which give rise to an expanded set of catalysts and to new metabolic reactions with new reaction products ($L_{x}$ to $L_z$). These in turn will recruit further transition metals into the metabolic system and so
The above-discussed hypothetical mechanism of evolution exhibits a form of autocatalysis by ligand feedback effect. At the same time and more importantly it also exhibits an avalanche self-expansion, which is not a feedback effect, because it leads beyond the established products of synthesis. Therefore, it is here termed “feed-forward effect”.

All chemical reactions are dependent on the reaction conditions. This dependence, however, is lessened more and more by more and more evolved metallo-catalysts compared to the de novo transition metal catalysts without the promotion by ligands. Therefore, every instance of a feedback or feed-forward effect means an incremental increase of independence from the environment, i.e. a deepening autonomy. The metabolism acquires an increasing propensity to run even under conditions, which no longer would allow its de novo initiation. This is how the metabolism becomes precarious, life becomes mortal, and chemistry becomes historic. Each additional feedback or feed-forward effect constitutes an instance of inheritance or memory and the process of evolution is seen largely as a concatenation of such memory effects.

Whenever the self-expanding metabolism brings forth a new branch pathway this will weaken to some extent the pre-existing metabolism, whence it branches.
If a product of the new branch pathway feeds back autocatalytically into this very same branch pathway, this weakening effect is amplified (“egotistic feedback”). However, if the product of the new branch pathway exhibits in addition to the egotistic feedback an “altruistic feedback”, i.e. a positive feedback effect or a feed-forward effect for the pre-existent metabolism, this compensates for the egotistic feedback. A reaction product with such a double feedback effect is termed “vitalizer”. Examples for vitalizers in extant metabolism are: coenzymes, ribosomes, protein translocases and nucleic acid polymerases.

The main features of the proposed mechanism of metabolic evolution are summarized in Table 1.1 in comparison to an RNA-implemented mechanism of evolution. A most significant difference is seen in the required persistence of a mutation. According to the RNA world theory any mutated RNA sequence must be stable enough to persist as a digital information storage medium over long periods of time. According to the iron–sulfur world theory a ligand feedback is not required to persist any longer than it takes for the next successor ligand feedback to emerge. The better ligand will wipe out its less efficient predecessor and so there is no need for persistence over long periods of time. This important point is sometimes not understood owing to an RNA world mindset (Blackmond 2009).

**Volcanic Flow Setting of the Pioneer Organism**

The Solar System started about 4.567 billion years ago. The Earth may have been cool enough for supporting liquid water as early as 4.4 billion years ago. At that time the partially parallel processes of accretion, core-mantel differentiation and crust segregation were still significantly incomplete (Kleine et al. 2002; Yin et al. 2002; Jacobsen 2003). This means that the juvenile Earth had a very hot magma
and a relatively thin, hot, highly reducing crust. The crust suffered heavy bombardment and impact cratering with intense fracturing and deposition of debris. As a consequence a large number of volcanic flow sites were available for the origin of life (Cockell 2006). These sites were characterized by chemically diverse flow channels for volcanic exhalations in combination with hydrothermal flows.

The volcanic flow setting may be described as follows: At very high temperatures the gas mixture in the volcanic exhalations is in chemical equilibrium. If the volcanic exhalations are cooled slowly, the gas mixture remains in chemical equilibrium. However, if the volcanic exhalations are quenched rapidly enough, the mixture of volcanic gases is “frozen” into a non-equilibrium state, i.e. it acquires a chemical potential. Quenching may occur in a variety of ways: Conduction cooling by contact with cold solids, internal cooling by mixing with cold water, melting cooling by contact with ice and/or evaporation cooling by pressure decrease.

In view of the highly reduced state of the crust the volcanic exhalations must have also been highly reducing. High molar ratios of CO/CO₂ would have existed in equilibrium at high temperatures. For example, under conditions of saturation with graphite the equilibrium molar ratio of CO/CO₂ of the system C–H–O increases with temperature and decreases with pressure and it is about 1:1 at 1,200°C and 2 kbar or at 900°C and 0.1 kbar (Holloway and Blank 1994). With decreasing temperature the water gas equilibrium,

\[ \text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + \text{H}_2, \]  

shifts in favour of CO₂. If quenching along the flow path is fast enough compared to the rate of the water gas shift reaction, a disequilibrium CO concentration arises providing not only a highly reactive carbon source, but also reducing potential for the pioneer organism,

\[ \text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + 2\text{H}^+ + 2\text{e}^-, \]  

akin to the carbon monoxide dehydrogenase reaction in biochemistry. In the metabolism of all extant autotrophs carbon dioxide is the dominant carbon source. This is the reason, why it was proposed as the primordial carbon source of life (Wächtershäuser 1988a, 1990, 1992) and this proposal was confirmed in conjunction with pyrite formation (Heinen and Lauwers 1996), even though chemical properties of CO₂ (insolubility of carbonates, thermodynamic stability of CO₂) seem to point to a later entry into the metabolism.

The water gas shift reaction generates dihydrogen (H₂), one molecule for each CO molecule that is oxidized to CO₂. This means that the water gas shift reaction replaces the reductant CO by the reductant dihydrogen (H₂). Dihydrogen is a prominent component of volcanic gases and it is produced by reaction of FeS and H₂S at the site of the pioneer organism or upstream thereof (Taylor et al. 1979; Wächtershäuser 1988c; Drobner et al. 1990). Dihydrogen has been suggested as possible reductant in a chemoautotrophic origin of life (Wächtershäuser 1988b, 1998a). It may generate electrons as in the hydrogenase reaction:

\[ \text{H}_2 \rightarrow 2\text{H}^+ + 2\text{e}^- . \]
In a volcanic flow system, HCN may be formed by reaction of CO with NH$_3$. This is suggested by industrial chemistry. The conversion is favoured thermodynamically by increasing temperature (Owen 1961). Ammonia in turn forms by nitrogen fixation, which is favoured thermodynamically by decreasing temperature:

$$\text{CO} + \text{NH}_3 \rightarrow \text{HCN} + \text{H}_2\text{O},$$  \hspace{1cm} (1.5)

$$\text{N}_2 + 3\text{H}_2 \rightarrow 2\text{NH}_3.$$  \hspace{1cm} (1.6)

Therefore, the equilibrium concentration of HCN at low temperature (e.g. 100°C) and low pressure would be quite low. However, aside from quenching effects the equilibrium may be shifted in favour of cyanide formation, if sequestration of cyanide by transition metals like Ni$^{2+}$ occurs. This reaction would form stable complex bonds, e.g. Ni–CN or Ni–CN–Ni. The Ni-centers are available as catalytic centers and the cyano ligands are available as C-source and N-source for the synthetic processes.

Volcanic exhalations may also comprise COS (Corazza 1986), which is known to form by reaction of CO with H$_2$S (Fukuda et al. 1977):  

$$\text{CO} + \text{H}_2\text{S} \rightarrow \text{COS} + 2\text{H}^+ + 2\text{e}^-. \hspace{1cm} (1.7)$$

COS may serve as source for group activation in the pioneer organism, but it hydrolyzes readily:

$$\text{COS} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + \text{H}_2\text{S}. \hspace{1cm} (1.8)$$

COS may also serve as carbon source, thereby generating activated thioacid groups (–CO–SH). COS also functions as intermediate for converting CO + H$_2$ + H$_2$S into methylmercaptan (Barrault et al. 1987), which is also found in volcanic gases:

$$\text{COS} + 6\text{H}^+ + 6\text{e}^- \rightarrow \text{CH}_3\text{–SH} + \text{H}_2\text{O}. \hspace{1cm} (1.9)$$

A hadean volcanic flow system may be conceptually subdivided into three flow regions:

1. A deep upstream flow region with a temperature above the critical temperature of water may be likened to a flow reactor with a stationary solid medium and a mobile gas phase in equilibrium.
2. A medium flow region with a temperature below the critical temperature of water may be likened to a trickle-bed reactor with a stationary solid phase, a volcanic gas phase and a mobile liquid water phase with dissolved volcanic exhalations. The water phase condenses progressively in gas flow direction. For its vital functions in nutrient conversions a thin transient film of water that merely wets the solid surfaces may be sufficient.
3. An uppermost flow region with a still lower temperature may be viewed as a chromatographic reactor with a stationary packed bed of solid debris or particles and a mobile liquid water phase.

Such a volcanic flow system exhibits temperature and pressure gradients as well as chemical composition gradients in flow direction. The third flow region may
exhibit chemical zoning (e.g. pH zoning or sulfidation zoning). Moreover, it may exhibit chromatographic characteristics for the organic products in the superstructure of the pioneer organism. This means that the constituents of the surface metabolism may have been selected by differential retention times in view of the state of volcanic flow. Reactive and chromatographic processes would have interacted along the flow path with best surface bonders being the slowest travellers. This renders the chemoautotrophic origin of life a spatially and temporally coherent, local affair as opposed to the global affair of the “prebiotic broth theory”.

A volcanic flow condition is of significance for the location of the onset of the pioneer organism by ligand-based feedback and fed-forward effects. We note that strong-bonding ligands have a long retention time and migrate slowly in flow direction. This means that they tend to remain concentrated in a relatively early and slow flow zone. This means in turn that stronger and stronger bonding ligands occur in ever more retarded flow zones, termed “ligand zones”. Thereby the pioneer organism becomes localized in spite of the volcanic flow condition.

Hydrolysis of orthosilicates in komatiite rock material is seen as the starting point for the formation of the inorganic substructure of the pioneer organism according to the following simplified, notional reaction formulae:

\[
\begin{align*}
(\text{Mg,Ca})_2\text{SiO}_4 + \text{H}_2\text{O} &\rightarrow (\text{Mg,Ca})(\text{OH})_2 + (\text{Mg,Ca})\text{SiO}_3, \\
(\text{Fe,Ni})_2\text{SiO}_4 + \text{H}_2\text{O} &\rightarrow (\text{Fe,Ni})(\text{OH})_2 + (\text{Fe,Ni})\text{SiO}_3.
\end{align*}
\]

The basic minerals \((\text{Mg,Ca})(\text{OH})_2\) serve as pH-buffer against acidic volcanic gases and acidic reaction products of the pioneer metabolism. Reaction with \(\text{CO}_2\) generates \(\text{CaCO}_3\) and basic \(\text{MgCO}_3\). \((\text{Fe,Ni})(\text{OH})_2\) undergoes subsequent alterations by dehydration, cyanidation, carbonylation, sulfidation and ultimately pyritization. Carbonylation products may mobilize transition metals as carbonyls (Cody et al. 2000) for subsequent transport through volcanic flow channels and bonding into or onto the substructure. Progressive sulfidation of \((\text{Fe,Ni})(\text{OH})_2\) generates \((\text{Fe,Ni})\text{S}\). \(\text{FeS}\) appears first as “amorphous” \(\text{FeS}\), which ultimately undergoes pyritization (1.1). An alternative source of electrons in the substructure of the pioneer organism is provided by the oxidation of ferrous hydroxide:

\[
3\text{Fe}(\text{OH})_2 \rightarrow 3\text{Fe}_3\text{O}_4 + 2\text{H}_2\text{O} + 2\text{H}^+ + 2\text{e}^-.
\]

By the above conversions the inorganic substructure offers not only a rich set of possible polynuclear, polymodal, heteroleptic structures exposed as catalytic centers in its surfaces, but also reducing power, e.g. for nitrogen fixation as demonstrated experimentally with \(^{15}\text{N}_2\) (Dörr et al. 2003):

\[
\text{N}_2 + 3\text{FeS} + 3\text{H}_2\text{S} \rightarrow 3\text{FeS}_2 + 2\text{NH}_3;
\]

or for the formation of methylmercaptan from \(\text{CO}_2\) with \(\text{FeS}/\text{H}_2\text{S}\) (Heinen and Lauwers 1996):

\[
\text{CO}_2 + 3\text{FeS} + 4\text{H}_2\text{S} \rightarrow \text{CH}_3\text{SH} + 3\text{FeS}_2 + 2\text{H}_2\text{O}.
\]
Experimental Synthetic Reactions

The above considerations give us a grasp of the possible energy sources, nutrients, reductants and physical conditions for a volcanic origin of life. Based on these insights we now turn to the problem of reconstructing the pioneer metabolism. This problem can only be solved by experiments and the theoretical-experimental research program has as its ultimate goal the reconstitution of an evolvable pioneer organism. This contrasts with the experimental program of the “prebiotic broth theories” that merely aims at a rationalization of some “prebiotic broth reactions” of an otherwise intractable process of early evolution. We chose the following aqueous, volcanic/hydrothermal reaction conditions:

*Temperature*: 100 ± 20°C, the temperature range of hyperthermophilic organisms.

*Pressure*: Autogenous pressure developed in a closed batch reactor. This pressure may be too low. The lack of a higher pressure for the acceleration of reactions may be compensated by increase of the concentrations of the reactants.

*Catalytic transition metals*: iron group transition metals: Fe²⁺, Co²⁺, Ni²⁺.

*Volcanic reactants*: CO, COS, HCN, NH₃, H₂S, CH₃SH.

*Reductant (electron source)*: Water gas shift reaction (1.3), pyrite formation (1.1).

*pH-buffer*: (Mg,Ca)(OH)₂, (Mg,Ca)CO₃.

**Activated Acetic Acid Thioester**

In extant acetyl-CoA synthase a Fe–Ni–S cluster catalyzes the formation of acetyl-CoA (CH₃–CO–S–CoA) from Ni-methyl, carbon monoxide and coenzyme A. It was retrodicted that activated acetic acid (CH₃–CO–SH or CH₃–CO–S–CH₃) may form from CH₃SH as methyl source and CO (Wächtershäuser 1990). This retrodiction was confirmed experimentally at 100°C for FeS, CoS, NiS, (Ni,Fe)S or Ni(OH)₂ as catalysts for a spectrum of slightly acidic to alkaline pH-values. If CH₃–SH is replaced by H₂S, the reactions proceed as well, whereby CH₃–SH is formed as intermediate from CO and H₂S.

These synthetic reactions provide a C₂-carbon skeleton and at the same time a portion of the chemical energy of the system is conserved in an activated carboxylic group (–CO–S–H or –CO–S–CH₃). The reaction must be seen as an organo-metal reaction with metal-carbon bonds (Huber and Wächtershäuser 1997). These group activations are available for further reactions and for transformation into phosphorylation energy (Wächtershäuser 1998a).
Pathways to α-Hydroxy Acids and α-Amino Acids

A series of ordered pairs of α-hydroxy acids and α-amino acids with the general formula R–CHA–COOH (A = OH, NH₂; R = H, HOCH₂, CH₃, C₂H₅, C₃H₇) was formed by reaction of a Ni²⁺-catalyst system with cyano ligands (from ¹³C–KCN) attached to Ni–centers (as carbon and nitrogen source), with CO (as carbon and electron source) in the presence of (Mg,Ca)(OH)₂ as hydroxy ligand source and as buffer. The presence of Fe²⁺, HS⁻, CH₃S⁻ ions does not disturb the reaction. It is noteworthy that α-hydroxy acids and α-amino acids are excellent ligands for transition metals. Moreover, pyruvate is formed as intermediate as well as glycol, perhaps via glycol aldehyde, the simplest sugar. Acetic, propionic and butyric acids are also found. Overall the product spectrum indicates a tendency of incremental chain extensions towards long-chain lipids, which perhaps is broken off early due to low pressure (Huber and Wächtershäuser 2006).

Activation of α-Amino Acids and Peptide Cycle

α-Amino acid activation and peptide formation were discovered when α-amino acids were reacted with CO plus H₂S (or CH₃SH) in the presence of NiS under alkaline conditions. Nascent COS appears to be the activating intermediate since its use instead of CO and H₂S (or CH₃SH) also produces peptides (Huber and Wächtershäuser 1998). Under the same conditions a peptide cycle was discovered (Huber et al. 2003). Peptides were found to react N-terminally with CO (or COS) to acquire an N-terminal hydantoin ring, which subsequently hydrolyzes in two stages, first to an N-terminal urea group and subsequently by urea cleavage. This reaction sequence constitutes N-terminal peptide elongation in competition with N-terminal chain degradation, each by one amino acid unit at a time.

In the first step of the peptide cycle an amino acid (aa) is apparently converted to the highly reactive aminoacyl N-carboxyanhydride (c). This means that the redox energy of reaction (c) is coupled to amino acid activation via COS, which may well be the simplest pioneer energy coupling of life. The free amino group of the dipeptide may react with another molecule of aminoacyl N-carboxy anhydride to form a tripeptide, which again may react by N-terminal chain extension to a tetrapeptide and so forth. A hypothetical mechanism of the entire peptide cycle is in shown in Fig. 1.6. Up to the stage of the urea derivative the peptide cycle is synthetic (anabolic). The subsequent hydrolysis is catabolic and reminiscent of the extant Ni-enzyme urease. The peptide cycle may be seen as a catalytic cycle for the conversion of CO to CO₂ akin to extant carbon monoxide dehydrogenase (CODH). The peptide cycle will run as long as the redox energy supply of CO lasts. The hydrolytically sensitive COS is merely an intermediate and its accumulation is not required. With additional α-amino acids a variety of peptides (and their hydantoins and ureas) are formed to coexist in mixture it constitutes a dynamic library, in which the peptides (hydantoins, ureas) come and go with steady state concentrations corresponding to...