Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 4283–4287, May 1994
Evolution

Life in a ligand sphere

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Communicated by Carl R. Woese, January 7, 1994

ABSTRACT **The most fundamental divide in biology is that between heterotrophic and autotrophic ways of life. Two of the leading proponents of a heterotrophic origin of life ("hetero-origin") in a prebiotic broth, C. de Duve and S. L. Miller, have criticized my theory of a pyrite-pulled chemo-autotrophic origin of life ("auto-origin") [De Duve, C. & Miller, S. L. (1991) *Proc. Natl. Acad. Sci. USA* 88, 10014–10017]. This criticism is now answered.**

The theory of a heterotrophic origin assumes a primitive ocean of slowly accumulating amino acids, bases, sugars, lipids, and other organic compounds. These are seen as self-organizing to the first reproducing entity. The chemistry of this speculative process is pictured along conventional lines: solution reactions with adsorption–desorption equilibria and heterogeneous catalysis on minerals. Over the past 60 years, these notions have come to be very deep-seated. This is perhaps the reason why the criticism by de Duve and Miller (1) is permeated with references to the principles of solution chemistry. "Wächtershäuser's theory," they write, "is imaginative and original, but none of it is plausible in the framework of aqueous solution chemistry." The adherence to this framework is unfortunate, for it has rendered some of their more interesting critical approaches inapplicable. Other criticisms, however, are relevant; and some are justified.

1. Methodology as Introduction

I have stressed repeatedly in my papers (2–4) my indebtedness to the scientific methodology of Karl Popper (5–8), which may be briefly characterized as theory-Darwinism. Theories are seen as competing with each other for survival *vis-à-vis* the facts. It is surprising that de Duve and Miller misunderstood my references to methodology "as an argument in support of validity" of my theory. Popper's methodology means a commitment to theoretical confrontation and competitive evaluation. The reward is not certain validity, but fruitful challenge. The commitment to the virtue of theoretical confrontation requires that theoretical conflicts are not smothered by any desire for compromise. Theoretical compromise leads to logical weakness. Applied to the present case, the clash between "hetero-origin" and "auto-origin" is crystal clear. For a hetero-origin, the concepts of a prebiotic chemistry and of a broth as an arsenal of organic building blocks are mandatory. For an auto-origin, the concept of a prebiotic chemistry never arises; and the primitive ocean, whatever its content, is irrelevant as an arsenal of organic building blocks for life. De Duve and Miller suggest implicitly a combination of the notions of hetero-origin and auto-origin by noting that "many of the products of [prebiotic broth] simulation experiments are carboxylic acids capable of anionically bonding to a positively charged surface, as required" by my theory. However, by combining the theory of an autotrophic origin with the tenets of the soup theory, we would obliterate for both theories the hallmark of their theoretical merit: explanatory power, the power to explain many facts with few assumptions (6).

2. The Energy Flow

All theories on the origin of life are faced with the problem of chemical consistency. Hetero-origin theories follow a tradition of "solving" this problem by the segregation of their prebiotic chemistry between a multitude of theaters with a diversity of reaction conditions and chemical potentials (9). Coming from this tradition of thought, de Duve and Miller raise the objection that my theory is weak on thermodynamic grounds for offering "only two sources of free energy to drive the metabolism of the surface biont: sulfide oxidation and anionic bonding." Here my critics are quite mistaken. As explained in detail in the next section, my theory offers not two energy sources, but only one. This is dictated by the logic of the situation of an auto-origin. All biochemical conversions in an autotrophic metabolism must occur in the same small locale. They must be driven by the chemical potential prevailing in said locale. This is the single-theater condition of my theory.

The energy source in my theory is a redox energy source: the reducing power of FeS/H_2S relative to oxidized carbon compounds (2). It is seen as producing pyrite, a deep thermodynamic sink, and reduced organic compounds en route to methane. Organic intermediates with anionic groups (e.g., $-COO^-$, $-S^-$, $-COS^-$) become bonded to the cationic surface of pyrite in *statu nascendi*. This establishes the first organized entity of life: a composite structure of a sphere of metastable organic ligands around a growing cluster of pyrite. Their anionic bonding provides a trough of metastability in the overall cascade of the redox energy flow—an intermediate within that energy flow, rather than the source and sink of the overall energy flow.

de Duve and Miller further object that "because of the lack of a coupling system the free energy of sulfide oxidation cannot be used to drive other processes, such as assembly reactions." This criticism is relevant, and, relative to my work that was published when de Duve and Miller wrote their criticism, it was also justified. However, subsequent developments of my theory have provided insights to this coupling problem.

The main pillars of my theory have been published, so far, in five installments. The first three refer to pyrite formation as the earliest energy source for life (2), the principles of a surface metabolism (3), and the first autocatalytic reproduction cycle (4). These have been considered and criticized by de Duve and Miller. Subsequently, a solution to the problem of the origin of biochirality was published (10, 11). A fifth installment (12) introduces a methodology for the retrodiction of archaic pyrite-pulled pathways from extant enzymatic pathways by elaborating the principles of the first three papers. It is in this latter paper that an archaic energy cascade from redox energy to group activation energy has been postulated, which may be represented notionally as follows:

$$HS\text{-}CH_2\text{-}COO^- + H_2S \rightleftharpoons {}^-S\text{-}CH_2\text{-}COSH + H_2O$$

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$$^{-}S\text{-}CH_2\text{-}COSH \xrightarrow[-FeS_2]{+FeS} {}^{-}|CH_2\text{-}COSH \xrightarrow{H^+} CH_3\text{-}COSH$$

This energy cascade has two remarkable features: (*i*) it generates carbanion activation not by a base, but by a redox reaction (ref. 12, pp. 129 and 134; ref. 13); and (*ii*) it generates thioacid activation, which later in evolution is replaced by thioester activation (ref. 12, pp. 129, 134, and 149).

3. Thermodynamic Aspects

de Duve and Miller claim to have detected in my theory "serious flaws of . . . thermodynamic nature" regarding the stability of mineral-bonded polymers. Their analysis assumes, however, an adsorption–desorption equilibrium in a soup of a monomer, capable only of weak bonding to a mineral surface. They come to the conclusion that under such conditions the chain growth of a surface-bonded polymer is unlikely. However, their premises are diametrically opposed to the principles of my theory. It should be clearly understood that in a pyrite-pulled chemo-autotrophic origin of life, the surface organism constitutes an irreversible flow-through reactor with (*i*) an input of inorganic nutrients; (*ii*) an "internal" surface metabolism of surface-bonded organic constituents, generated on the surface and adhering as ligands to the surface; and (*iii*) an output of surface-detached organic products of decay. These products of decay disappear irreversibly because of diffusion. The "internal" surface metabolism may be partly irreversible and partly close to equilibrium (see ref. 3, pp. 454–455, 465–466; ref. 4). I will here distinguish three different cases.

(*i*) A strongly surface-bonded polymer A^s_n is formed directly and irreversibly by an autotrophic process. It cleaves irreversibly into surface-bonded monomer ($A^s_n \rightarrow A^s_{n-1} + A^s$). The monomer is a weak surface bonder. It detaches rapidly (short residence time) and disappears irreversibly by diffusion ($A^s \rightarrow A$). In this case the polymer will exist as a steady-state intermediate. Its (small) concentration will depend on the relative rates of autotrophic polymer generation and polymer cleavage.

(*ii*) Now we modify the above case by assuming a surface equilibrium between the polymer and its monomer ($A^s_n \rightleftharpoons A^s_{n-1} + A^s$). The monomer detaches from the surface and disappears irreversibly by diffusion. The steady-state concentration of the polymer will depend on the relative rates of autotrophic polymer generation and monomer detachment and on the constant K^s of the surface equilibrium. It will be high if K^s favors the surface-bonded polymer and/or if the monomer is a strong surface bonder with a long residence time.

(*iii*) On the other hand, surface-bonded monomer may be assumed to be formed directly by an irreversible autotrophic process and to exist in a surface equilibrium with its polymer. The monomer detaches from the surface and disappears irreversibly by diffusion. In this case again the concentration of the polymer will be high if the constant of the surface equilibrium K^s favors the surface-bonded polymer and/or if the monomer is a strong surface bonder with a long residence time.

In cases *ii* and *iii* the surface equilibrium is decisive. It will favor the surface-bonded polymer if the standard free energy ΔG^s of surface polymerization $A^s_{n-1} + A^s \rightarrow A^s_n$ is negative. Ultimately, this value will have to be determined experimentally. However, a rough estimate is possible by transforming the fundamental equation of thermodynamics of a solution polymerization ($\Delta G = \Delta H - T\Delta S$) into that of a surface polymerization ($\Delta G^s = \Delta H^s - T\Delta S^s$). In cases where the surface-bonding anionic group is separated from the polymerization (or polycondensation) group, we may simply assume $\Delta H = \Delta H^s$. As an example, let us now assume $\Delta H < 0$, $\Delta S < 0$, and $|\Delta H| < |T\Delta S|$. This means that the solution equilibrium disfavors the polymer. In the corresponding surface reaction, the absolute value of the entropy of reaction is smaller ($|\Delta S^s| < |\Delta S|$) due to the fact that there is less change of mobility by reaction on the surface. Such a reaction is quasi-intramolecular. This entropy effect of the surface metabolism holds for all reactions. If it is sufficiently pronounced, the relation $|\Delta H^s| > |T\Delta S^s|$ may hold, which means that the polymer is favored on the surface while disfavored in solution. de Duve and Miller obviously have an adequate intuitive grasp of this situation, for they admit elsewhere in their paper: "The equilibria for the formation of hemiacetal polymers are not particularly favourable in aqueous solution, but the surface may change this."

A strong surface bonding of the monomer has the effect of providing a degree of thermodynamic isolation of the surface metabolism by the inhibition of detachment. By assessing the bonding strength based on well-known laws of physical chemistry, I proposed the exclusion rule that the organic constituents must be anionic (e.g., $-COO^-$, $-S^-$, $-COS^-$, $-OPO_3^{2-}$), while the mineral surface must be cationic. Pyrite satisfies this requirement because of exposed ferrous ions. Now, an ionic bond has always a covalent component, notably if the ions are soft. This is basic chemical knowledge. de Duve and Miller have failed to apply it. They speak instead of binding "by purely electrostatic interactions" and of "binding surfaces . . . provided by a simple charged plate." None of these terms or concepts has been used by me, nor should they be used by any other chemist. Rather, strong-bonding constituents should be seen as a ligand sphere around a pyrite cluster.

de Duve and Miller's reliance on "purely electrostatic interactions" leads them to criticize the surface metabolism as being too much like "random chemistry" to enable a complex metabolic network. Elsewhere, however, they take the other extreme point of view. "One does not see," they write "what could prevent the organism from evolving into a dead end, in which the surface is covered by molecules so tightly bonded that they cannot be further displaced." However, I have suggested (3, 4) how such a dead end in evolution is avoided: by the evolutionary conversion of anionic foot groups of surface-bonded lipids into nonbonding groups. This in fact is a central point in the model of cell evolution within my auto-origin theory.

4. The Evolution of Pathways

My theory proposes an overall evolution in three stages. The first is that of an open surface metabolism. The second stage is the semicellular stage with a closed membrane envelope (membrane metabolism), internal pyrite (surface metabolism), and a cytosol (cytosol metabolism). The third stage is that of a true cell without internal pyrite (ref. 3, p. 463). Surface-bonded catalysts, notably anionic noncoded peptides, are seen as appearing in the first stage (ref. 3, p. 479). The genetic machinery is seen as appearing in the second stage (ref. 3, p. 453), and its coded enzymes (if anionic) will be bonded to the pyrite surface (ref. 3, p. 479). Now, it is important to understand that the evolution of the archaic biosynthetic pathways occurred parallel to this overall process. The surface metabolic phase of pathway evolution begins in the first stage and extends into the second, while the evolution of membrane and cytosol pathways begins in the second stage and extends into the third. This means that the archaic pathways come increasingly under the influence of surface-bonded catalysts: precursors of coenzymes, peptides, and enzymes.

de Duve and Miller did not fail to recognize this (ref. 1, p. 10015, left column last paragraph): "The author does bring in catalytic help in the form of anionically bonded molecules."

But why then did they write elsewhere (ref. 1, p. 10015, right column, penultimate paragraph) "the pathways of Wächtershäuser's model are considered entirely determined by the peculiarities and opportunities provided by anionic bonding"? This is a serious and unfortunate misreading of my work, for it lures de Duve and Miller into the extraordinary belief that the surface-bonded pathways (3) have been proposed to proceed, not with the help of surface-bonded catalysts or enzymes as I suggest, but "entirely determined . . . by anionic bonding to the pyrite surface." They then hasten to note that nothing known "about surfaces suggests that they have the extraordinary organizing power attributed to them in Wächtershäuser's theory."

Any theory of early evolution should have as its main purpose the problem of explaining biochemistry. This means that the principles of such a theory should be useful for retrodicting archaic pathways. I have followed such a program of retrodiction. In my paper on the principles of a surface metabolism, I have used the capability of anionic surface bonding as the main principle of retrodiction. In my paper on the first autocatalytic cycle (4), I have brought in additionally some principles of sulfur and pyrite chemistry to retrodict an archaic carbon-fixation cycle. However, a full-fledged general methodology of retrodiction has been introduced initially only in 1992 (12). Within this methodology further progress is expected. For example, I now suggest, as a further modification of my recent proposal (12), that the evolution of purine biosynthesis begins with a carbon-fixation reaction producing first uric acid, the most oxidized purine.

5. The Genetic Machinery

Perhaps the most crucial problem of pathway retrodiction is concerned with the origin of the genetic machinery. The theory of a pyrite-pulled auto-origin promises a solution of this problem. The appearance of the genetic machinery presupposes the appearance of a nucleic acid, which in turn presupposes the appearance of phosphorylated sugars. Within my theory I have proposed a model for this evolution. It assumes an archaic form of a nucleic acid (TNA) in which the purine bases are attached glycosidically to a polyhemiacetal backbone of surface-bonded triosephosphates. de Duve and Miller have made an interesting observation regarding this hypothetical structure. They assume that it is in a rapid equilibrium of formation and decomposition; therefore, they conclude that it cannot hold "any genetic information."

In my papers I have deliberately avoided the use of the "information" metaphor because of its concept-narrowing effects and because it is not needed in chemistry.* In chemical terms the process of reproductive multiplication is a synthetic chain reaction with branching, which may be represented as an autocatalytic production cycle. From this vantage point the mechanism of evolution may be given a very simple formulation: the appearance of branch products with a dual catalytic feedback—a feedback into the production cycle and a feedback into their own branch pathways (3, 12, 14). This is evolution by autocatalytic expansion loops. Each expansion loop is induced by the low-propensity *de novo* formation of a catalytic branch product. It latches onto the production cycle by the autocatalyzed high-propensity formation of the catalytic branch product. In conventional chemical terminology this is called a memory effect. It is the physical basis for heredity. The information metaphor is neither needed nor helpful for an understanding of this mechanism.†

Within such a theory of evolution, multistep models for the appearance of nucleic acids and the genetic machinery can be constructed. In one such model (3), the bases (e.g., imidazoles) of the earliest TNA would be acid–base catalysts not involving base pairing. In a second step, base-pairing catalysis of TNA would arise. It would not require, however, any special sequences for folding. Any sequence (even a homopolymer) determined by thermodynamic stability may be sufficient. For such a sequence, the problem that it might "dissolve away," as de Duve and Miller put it (incidentally, the standard problem of all RNA world models), simply would not exist.

The prebiotic broth is pictured as a broth of ready-made modules for biosynthesis. Therefore, within hetero-origin theories it is commonly assumed that life must start with a modular metabolism. Coming from this background, de Duve and Miller assumed that the hypothetical TNA structure could only be formed by a reversible modular process. This assumption does not apply to the content of my papers. I have stressed that, by the logic of an auto-origin, the early forms of metabolism cannot be modular. They must proceed piecemeal, and this must hold also for TNA (ref. 3, pp. 468 and 470).

6. Carbon Fixation

By definition a theory on the auto-origin of life has to be a theory of carbon fixation. Therefore, I have written extensively on this issue (2, 3, 4, 12, 15), and I have devoted one paper exclusively to the evolution of carbon fixation from an archaic, pyrite-pulled version of the reductive citric acid cycle (4). In that paper I have made detailed predictions of pyrite-pulled reactions. de Duve and Miller have noticed and referred to that paper. Yet they write: "In contrast [to the soup theory], Wächtershäuser offers no explanation for the formation of the primary building blocks."

By my theory the early evolution of life may be largely seen as an evolution of carbon-fixation pathways. The acetyl-CoA pathway and the glycine synthetase pathway are carbon-fixation pathways. They are anaplerotic to the reductive citric acid cycle, and an archaic version thereof may initiate an archaic version of said cycle. Moreover, the purine pathway derives from these pathways as yet another CO_2-fixation pathway. From the vantage point of an origin of life in an archaic version of the reductive citric acid cycle, the appearance of the sugars, notably the triosephosphates, constitutes a fascinating problem, since the sugars are thermodynamically steeply uphill from pyruvate. One possibility of solving this problem would be to assume that prior to the "invention" of TNA, the surface organism had to "invent" a carbon-fixation pathway to the triose phosphates (akin to the Calvin cycle). Therefore, I wrote, "As a point of departure [for the discussion of the evolution of the genetic machinery] we assume that in an early stage of surface-metabolic evolution, surface-bonded C_3-units (notably phosphotrioses) arise by an unknown carbon fixation mechanism through thioacid activation" (ref. 3, p. 470). Later (12) I have proposed another possibility: the phosphorylated sugars may be the evolutionary successors of an earlier thiosugar metabolism, since thiotrioses are thermodynamically downhill

*The information metaphor is occasionally used in the chemical literature to express that two molecules have the "information" for reacting with each other. In chemistry such usage has no information value.

†In the origin-of-life field, the use of the information metaphor has led to the unfortunate prejudice that life must have started with a polymer sequence. To undermine this prejudice I have resorted occasionally, in replies to questions after lectures, to the distinction between analog information and digital information. By my theory, life would then have started with analog information, and it would have "invented" digital information later.

from pyruvate. But at least in detail the problem is still largely unsolved. de Duve and Miller would have been justified to point this out. But instead, they used the above quote out of context to support their allegation that my theory "offers no explanation for the formation of the primary building blocks."

7. Predictions and Corroborations

de Duve and Miller end their paper on a positive note: "If things are different on [pyrite] surfaces, then this should be demonstrated experimentally . . . Striking results might force us to change our opinion. The time is now ripe for the demonstration of one or two of the many provocative surface-catalyzed chemical syntheses proposed by Wächtershäuser." This call for experiments is wholly justified. I will present my reply in a numerical order, beginning with experiments which have been carried out in cooperation with K. O. Stetter and his coworkers.

(*i*) The possibility of an oxidative formation of pyrite is a centerpiece in my theory. It was in conflict with the conventional theory that under geochemical conditions pyrite can only be formed by a nonredox reaction from iron sulfide and elemental sulfur (16). In regard to this conflict, we conducted an *experimentum crucis* (17). It showed that under strictly anaerobic and geochemically plausible conditions, molecular hydrogen and pyrite are formed from iron sulfide and hydrogen sulfide. de Duve and Miller have objected that "the efficiency of the process seems low." They have failed to appreciate, however, that a certain inhibition of this reaction is precisely what is called for by my theory (4). If this reaction were uninhibited, the reducing potential of FeS/H_2S could not persist to drive a metabolism.

(*ii*) From the proposal of an archaic pyrite-pulled reductive citric acid cycle, it was predicted that α-ketocarboxylic acids should be reduced by FeS/H_2S to carboxylic acids (4). We corroborated this prediction by converting phenylpyruvate into phenylpropionate and cinnamate, both hitherto unknown chemical reactions (18).

(*iii*) It was further predicted (4) that the conversion $-CH_2-SH \rightarrow -CH_3$ by means of FeS should be possible. This prediction was confirmed by the conversion of $HS-CH_2-COOH$ to $H_3C-COOH$ (18).

(*iv*) By testing for the reaction mechanism of the above reactions, we found a surprising reaction type of FeS/H_2S: the reductive elimination of $HS-CH_2-CH_2-X$ (X = OH, SH, NH_2) to ethylene (18).

(*v*) It was suggested that the evolution of the Fe-only nitrogenases may go back to an archaic metabolism in an iron–sulfur world (3, 4, 12). We have begun testing this proposal by using acetylene as a model compound. Both ethylene and ethane were formed with FeS/H_2S (18).

(*vi*) We further made the surprise finding that nitrate is reduced by FeS/H_2S to NH_3 in a rapid reaction (18).

(*vii*) The prediction of energy coupling (12), referred to above in Section 2, has been tested by successfully trapping the presumptive thioacid intermediate. With aniline or amino acids, *N*-acetyl derivatives were formed (19).

(*viii*) It was predicted from the proposed pyrite-pulled origin of life in conjunction with a principle of mechanistic continuity that extant redox enzymes with iron–sulfur clusters should exhibit disulfido ligands in their clusters at least transiently (2, 12). This prediction was corroborated by the discovery that the P-cluster in nitrogenase contains actually a minimum pyrite cluster with a disulfido ligand, whereby each of its two sulfur atoms is coordinated with three iron atoms (20).

(*ix*) Since a great variety of geochemical redox energy sources are exploited by one microorganism or another, it was suggested that there may be still bacteria or archaea with metabolic reactions supported on internal pyrite (2, 3). This prediction received a hint of corroboration by the discovery of bacteria with internal pyrite grains (21).

(*x*) It was suggested that nucleic acid precursors, like TNA, should have an all-purine structure (3, 12, 22) with nine-bonded and three-bonded purines. It had been predicted that three-bonded purines would not fit into an RNA structure (22). This was corroborated by Orgel and coworkers (23).

8. Methodology as Conclusion

The debate of determinism vs. indeterminism clearly belongs to the realm of metaphysics (24). But reflection on this issue has proven to be of great value in dealing with a crucial problem in the construction of my theory. In my writings (3, 4, 12) I have tried to be clear and explicit on this point. Yet, de Duve and Miller found my explanations confusing and "somewhat ambiguous." I therefore feel obliged to give some further explanations.

The position of physical determinism means for chemistry that the reactive behavior of individual molecules cannot be predicted to an unlimited degree. However, experimental chemistry is concerned with the reaction of very large ensembles of molecules. Chemical laws regarding the reaction of such ensembles are therefore statistical laws. This is the basis for chemical reproducibility. de Duve identified this kind of statistical reproducibility with determinism (25).

An autocatalytic feedback cycle may become ignited with the *de novo* appearance of a single catalytic molecule (see Section 6). Such a singular ignition may have a nonreproducible induction period. After the ignition event, however, the catalytic molecule will become multiplied to a large ensemble by the operation of the autocatalytic cycle. Therefore, the operation of the autocatalytic cycle will be a reproducible affair. According to my theory, the early CO_2-dependent autocatalytic cycles are based on low-molecular catalytic molecules. The organic chemists will appreciate that there must be a paucity of possibilities for the first autocatalytic cycle and for its early autocatalytic expansions. In the most extreme case there may be only one chemical possibility for such a first cycle and for the early sequence of expansions of this cycle. In this sense it may be considered chemically determined. All of the ignitions, however, of the first cycle and of its early expansions may have unpredictable induction periods.

Now, it is important to realize that a synthetic autocatalytic cycle has an inherent tendency to evolve toward higher complexity. This means an inherent tendency toward an increasing number of reaction possibilities. Thus, life creates its own prospects. This is the physical basis for the interpretation of evolution as a process of self-liberation.

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