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Autopoietic Gene-Enzyme Cycles and the Emergence of Life

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Dr. LaViolette has been president of the Starburst Foundation since 1984, where he has conducted interdisciplinary research in physics, astronomy, geology, climatology, prebiotic evolution, psychology, and cultural anthropology. He received his BA in physics from Johns Hopkins, his MBA from the University of Chicago, and his Ph.D. in systems science from Portland State University. He has conducted occupational safety research at Harvard University, and served as a solar energy consultant to the Club of Rome and Greek government. He has also consulted Hughes Aircraft on ways to improve company innovation. In 1974 he developed a thermodynamics of market behavior and demonstrated that stock price bubbles exhibit predator-prey oscillation characteristics. He is originator of subquantum kinetics, a new theoretical approach that places physics and astronomy on a general systems theoretic footing and whose predictions have had over 12 confirmations. He also is originator of the galactic superwave theory, the notion that cosmic ray outbursts from a galaxy's supermassive core travel rectilinearly outward through the galaxy at close to the speed of light. In testing whether past superwaves have affected the Earth's climate, he became the first to discover high concentrations of cosmic dust and gold in ice age polar ice. He is author of six books, which include *Secrets of Antigravity Propulsion*, *Genesis of the Cosmos*, *Subquantum Kinetics*, *Earth Under Fire*, *Galactic Superwaves*, and *Decoding the Message of the Pulsars.*

Abstract

Systems concepts are applied to solve the problem of how early life could have emerged from an initially abiotic organic environment. Proteinoid or lipid microspheres are proposed to have polymerized from a primordial organic soup and to contain various amino acids and several different nucleobases. A self-replicating "basic set" hypercycle consisting of 10 XNA gene strands and 10 enzymes is proposed that utilizes inorganic phosphates as an energy source. The genes would utilize triplet combinations of adenosine and uracil to code for a replicase enzyme, a polymerase enzyme, and eight code translator (synthetase) enzymes. It is shown that there is a high probability that the basic set genes would emerge. Fissioning of the basic set microspheres into a population of microspheres all containing the basic set, could eliminate the problem of a single gene monopolizing use of the replicator enzyme at the expense of the others and greatly enhance the survivability of the replicating population as a whole. A thermodynamic analysis of such a self-replicating system is also presented. It is shown that genetic mutations will in the long-run allow the basic set to evolve to increased diversity, higher rates of enzyme synthesis, and greater rates of entropy production. Long-term evolution could have resulted in organisms similar to contemporary bacteria that utilize RNA genes with a four nucleobase codon system.

Keywords: prebiotic organic synthesis, proteinoid microspheres, lipid microspheres, autopoietic system, nonequilibrium systems, order-through-fluctuation, dissipation, XNA replication, hypercycle, biological evolution

1. Introduction

The following paper attempts to show that life-like self-replicating protobionts could have emerged in a plausible way as a consequence of physical processes taking place in nature. We begin by examining how an organic soup of amino acids and nucleobases could have emerged on the early Earth through natural terrestrial processes as well as through the influx of extraterrestrial organic material. We then consider how proteinoid microspheres would have naturally developed in an amino acid broth having properties similar to primitive cells. Next we consider how a simple self-replicating set consisting of 10 proto gene strands and 10 enzymes could have developed within a microsphere as a result of all ingredients fortuitously being present in close proximity. We calculate the probability of a given microsphere containing the proper ingredients for autopoietic self-replication of its contents and find that, in view of the large number of microspheres present in the protobroth, the formation of such a basic set would have been highly probable. We find that the principle of order-through-fluctuation devised by Ilya Prigogine to explain the emergence of order in nonequilibrium chemical reaction systems applies quite well to this prebiotic evolution model. However, the ordering phenomenon exhibited by self-replicating systems is of a distinctly different variety from that of reaction-diffusion systems. We compare these two general ordering phenomena and examine how they are different. We then show how thermodynamic concepts pioneered by von Bertalanffy and Prigogine may be applied to the order increasing ability of protobionts. We also find that terminology developed by the Brussels group for application to nonequilibrium systems may be applied to describe protobiont evolution. We find that gene mutations cause the self-replicating gene-enzyme set to evolve toward more efficient biosynthesis and increased rates of thermal dissipation. In conclusion, we examine how these proteinoid microspheres with their self-replicating gene-enzyme contents would have evolved into modern cells.

2. Prebiotic organic synthesis

The evolution of life is thought to have begun about 4.6 billion years ago around the time of the formation of the earth. Early theorists suggested that the Earth's primeval atmosphere consisted of methane, hydrogen, ammonia, and water with some H_2S creating a strongly reducing environment. They suggested that methane, ammonia, hydrogen, and water in the atmosphere reacted under the action of natural geological and cosmic energy-providing processes such as heat, lightning, shock, UV, and particle radiation to produce a wide range of small organic molecules (Oparin, 1924; Toupance, 1971, p. 83; Hochstim, 1971, p. 96). These monomers (i.e., amino acids, fatty acids, nucleotides, and sacharides), it was suggested, would have literally rained out of the sky covering the earth's surface with proteinoid and lipid material which became concentrated in the oceans together with other products.

More recently the alternative theory was put forth, based on harder evidence, that the Earth's atmosphere was instead mildly reducing and was made up primarily of carbon dioxide, nitrogen, with some water. Plankensteiner et al. (2004, 2006) began with a neutral atmosphere composed of carbon dioxide, nitrogen, and water vapour above liquid water simulating a primordial lake or ocean and subjected it to electric discharge. They found that this produced a variety of amino acids under a range of temperature conditions. The amino acids would not have suffered significant deterioration since the atmosphere at that time would have been mildly reducing.

Also Rode, et al. (1980) discovered the salt-induced-peptide-formation (SIPF) reaction in which they were able to form peptides from amino acids present in a hot, salty aqueous

environment containing a trace of copper ion (Cu^{II}). They found that it worked for all varieties of amino acids in solutions of various concentrations, making it a viable possibility for how peptides could have formed on the primordial earth. Experiments conducted by Dubina, et al. (2013) indicated that potassium chloride, rather than sodium chloride, was a better polymerizing agent for this process. They suggest that Na^{+}, Mg^{2+}, and Ca^{2+} may have been involved in later stages of peptide evolution, and that other inorganic cations, or clays or minerals may also have played a role.

Earlier Fox (1971a, p. 10; 1972, p. 26) had suggested that amino acids might have polymerized in geothermal regions near volcanic activity, where pools of water containing monomers could have become evaporated and reached temperatures over 100° C. He suggested that in the resulting anhydrous state monomers would have bonded to form polymers such as proteinoids, lipids, polynucleotides, and polysacharides. However, Rohlfing (1976) later demonstrated that amino acid broths were able to form polymers even at temperatures as low as 65° C when heated for up to 3 months. Such conditions would have been much more prevalent on earth since they are known to be generated today in certain terrestrial environments through solar heating.

A key hurdle to explaining the origin of life is to account for the formation of nucleobases since these are not as easily fabricated as amino acids. The autopoietic biosynthesis scheme suggested later in this paper requires the preexistence of at least two nucleobases in the prebiotic soup, e.g., adenine and uracil. Saladino, et al. (2001) have shown that it is possible to synthesize the nucleobases adenine and cytosine from formamide in the presence of appropriate catalysts such as $CaCO_3$ and various inorganic oxides, namely silica, alumine, kaolin, and zeolite. Also uracil type components have been found in volcanic eruption products (Podkletnov and Markhinin, (1981).

The other possibility is that nucleobases and amphiphilic compounds may have entered the Earth's atmosphere from space due to the continuous influx of cosmic dust and meteorites. Comets and asteroids containing carbonaceous products may have been another source. The purines adenine, guanine, hypoxanthine, and xanthine, as well as the pyrimidine uracil have been found in extracts from the Allende carbonaceous chondrite (Stoks and Schwartz, 1981). Also uracil has been found in extracts from three different carbonaceous meteorites from Murchison, Murray, and Orgueil (Stoks and Schwartz, 1979). A diverse suite of nucleobases have been found in the Murchison and Lonewolf Nunataks carbonaceous meteorites (Callahan, et al., 2011). In addition, radio astronomers have found that tiny ice grains in giant interstellar gas clouds contain cyanomethanimine, a chemical intermediate in the formation of adenine (Zaleski, et al., 2013). Finally, Deamer (1985, 1986) has found that nonpolar organic substances extracted from the Murchison carbonaceous chondrite produce cellular structures having boundary layers resembling cellular membranes and suggests that meteorite infall may have been an important contributor of such substances to the prebiotic environment.

3. The formation of microspheres

It has been observed that in an aqueous medium proteinoid material will aggregate to form cell-like structures called *proteinoid microspheres*; see Fig. 1. The work by S. Fox and others on proteinoid microspheres has stirred much interest because it appears that these chemical microsystems display many of the properties characteristic of cellular life (Fox, S., 1971a, p. 13; 1971b, p. 51). For example, they are open systems: they aggregate newly synthesized proteinoid material from their environment, grow in size, and replicate by growing buds and

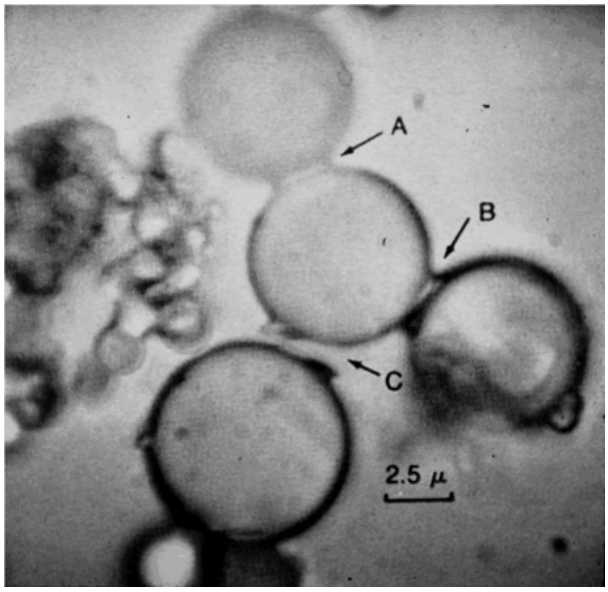

Fig. 1. "Budded" proteinoid microsphere; arrows indicate points of fusion (Sydney Fox).

fissioning in a manner similar to yeast. They have a uniform size in the range of cellular sizes. They have weakly catalytic properties. Their outer shell has membrane-like characteristics such as the ability to selectively transfer molecules. Finally, proteinoid microspheres have a tendency to associate with each other and to sometimes fuse, exchanging their contents (Hsu, 1972).

The ordering of proteinoid material into microspheres by accretion is an entropy-driven process ($\Delta S_T > 0$), rather than a free-energy driven process ($\Delta G < 0$) (Jungck, 1972, p. 103; R. Fox, 1972, p. 94). That is, the heat released in the process of accretion is so small that the observed behavior (an apparent decrease in positional entropy of the polymer) cannot be accounted for solely in terms of free energy. A closer look, however, reveals that the optically observed decrease in positional entropy is only apparent. Water molecules formerly attached to the proteinoids are released in the aggregation process leading to a net increase in entropy (for the system + environment). Thus, there appears to be a redistribution in entropy, a randomizing of the water structure to compensate for the configurational ordering of the associated polymers. Entropy-driven ordering is quite common in biochemical systems. Examples include the homopolymerization of tobacco mosaic virus A-proteins to form TMV rods, the hydrolysis of ATP to form ADP, the "self-assembly" of ribosomes, membranes, and fibrin clots, and many others. Furthermore Nakashima and Fox (1980) found that lysine-rich proteinoids of basic pH in aqueous solution catalyze the formation of peptides from free amino acids and ATP. Peptide production was optimal when the proteinoid pH was around 11 and ambient temperature above 20° C. They suggest that pyrophosphate could be used instead of ATP with lesser yield rate results.

As an alternative protocell, researchers are also considering visicles bounded by fatty-acid membranes formed from abiotically generated amphiphiles or lipid (Schrum, et al., 2010; Budin, et al., 2012). Studies have shown that such lipid protocells would have been more permeable to the diffusion of nutrients from their environment and would have more readily grown and subdivided. Whether these protobionts evolved from proteinoid or lipid microspheres, the discussion below should apply equally to either case. For the sake of economy, however, we continue this discussion by referring to their enclosures as being proteinoid microspheres.

4. Emergence of the basic set hypercycle

Now imagine the earth in the prebiotic period -- its oceans, lakes and geothermal pools filled with communities of polymers and proteinoid microspheres interfused with and bathed in a monomer broth. This was the "primordial soup", a mass of seething activity. New microsphere polymer combinations would be continually arising: a) as new microspheres would nucleate from the proteinoid broth and as old microspheres would dissociate, b) as existing microspheres would grow by accretion and fission into separate units, c) as microspheres would conjugally join with one another, and d) as the material within individual microspheres would become transformed through the action of internal catalytic reactions and cosmic ray radiation.

Because of the great variety of proteinoids and the (at least) two nucleobases present in the broth, it was more likely than not that each individual proteinoid microsystem would contain a different set of molecular combinations. And, because of the rapid turn over and intermixture, new sets of combinations would be constantly evolving. Moreover, each gram of proteinoid material would have contained on the order of a billion molecular microsystems. It is conceivable that sooner or later a special nucleobase and polypeptide combination could have arisen that no longer behaved as an aggregate but instead showed specific systemic properties, more specifically, a combination which behaved as a *self-producing* system. This special combination or *basic set*, as it will be called, would have exhibited *autopoietic* behavior, to use the general term suggested by Maturana and Varela (Maturana and Varela, 1973; Varela, Maturana, and Uribe, 1974). That is, the components of the basic set taken together as a unity, would have constituted a production network whose products were functionally identical with the components of the basic set. In other words, the basic set polymers functioning together as a system would have been able to catalyze the polymerization of new basic set polymers from monomers that diffused into the microsphere from the surrounding broth. Amino acids depleted within the microspheres due to synthesis of the basic set elements would be continually replaced from the broth by diffusion. It is conceivable that this internal "protein synthesis" polymerization process would take place at a rate several orders of magnitude greater than that of growth by external accretion of proteinoid material. Consequently, a microsphere containing basic-set gene strands and enzymes would rapidly grow and subdivide forming a community of protobionts. As these primitive life forms multiplied, they would eventually have predominated over the non-living (nonautopoietic) microspheres.

As a demonstration that the emergence of a basic set would have been highly probable, consider the following example. Suppose that this early broth only contained the nucleobases adenine (A) and uracil (U) and that rather than forming a helix, as in contemporary genes, they instead linked together as a linear chain. Moreover suppose, as Mednikov (1971, p. 425) has suggested, that the backbones of these primitive genes were neither made of ribose or of deoxyribose sugars but rather were made from a mixture of these and possibly even contained other sugars. We may call this genetic material "XNA" rather than RNA or DNA. Since A and U are complements, according to the Watson-Crick pairing rule, given the proper enzyme catalysts, such genes would have been capable of reproducing themselves (via their complementary strands) by template action.

Let us suppose that the XNA gene strands were composed of a series of codons, each composed of three nucleobases. A sequence of many such nucleobase codons would have coded the amino acid synthesis of a particular enzyme. Each XNA strand may have been composed of

as many as 300 nucleobases. Now, in order for a set of XNA gene strands to be able to reproduce themselves in an exponential manner, they would have had to contain the information necessary to synthesize their own protein replication catalyst which will be called XNA replicase. However, to effect the synthesis of XNA replicase, translator enzymes would have been required which would have been capable of translating information coded on the replicase gene strand into corresponding sequences of amino acids which would have in turn linked up linearly as a chain and finally folded up to form the XNA replicase enzyme. To ensure that there are an abundant supply of these translator enzymes, corresponding gene strands would have been required to code their production. The number of translator enzymes required would depend on the number of different codons on the gene strand. If the codons consisted of triplet combinations of A and U nucleobases, eight different triplet nucleobase combinations would be possible, hence eight different codons, each coding for a particular amino acid. Consequently, eight different translator enzymes would have been required.

Polypeptide assembly would not necessarily have required a ribosome, but could have been conducted directly on the XNA strand by successive visits from translator enzymes; see Fig. 2. These translator enzymes may have been similar to the present day amino-acyl synthetases, or "activating enzymes." Activating enzymes in contemporary cells function catalytically to link up transfer RNA's with the proper amino group for use in the ribosome. These prebiotic translator enzymes would have contained paired recognition sites so that they could pair up a particular amino acid to a particular nucleobase codon.

Finally, a "polymerase" enzyme would have been required to polymerize the individual linearly aligned amino acid sequences into a polypeptide chain. Thus, in addition to the gene coding for the replicase enzyme, the eight genes required to code for the synthetase-like translator enzymes, a tenth gene would have been needed to code for the assembly of the polymerase. Altogether 10 genes and 10 enzymes would have been required. This group of 20 would constitute the *basic set*. This is proposed as the minimal gene-enzyme set needed to produce life-like replication behavior.

Both the polymerase and replicase enzymes might have utilized naturally occurring inorganic phosphates as an energy or negentropy source (Kulaev, 1974, p. 271; 1971, p. 458; Swartz, 1972, p. 129; Miller, 1971, p. 83; Baltscheffsky, 1971, p.466). Fox (1972, p. 91) has suggested that fluctuating temperatures are able to serve as a means of chemically "trapping" free energy in a metastable state (as polymers), a phenomenon known as temperature-shift free energy generation. He suggested that similar processes could have produced energy-rich phosphates from naturally occurring orthophosphoric acid which could have served as energy reserves for catalyzing primitive biochemical reactions.

This overall process of reproduction and protein synthesis is described in Table I. The same set of eight translator enzymes (synthetases) could have been used as a "universal" genetic code

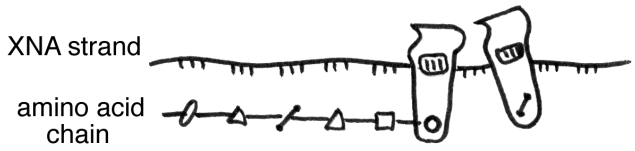

Fig. 2. Synthetase translator enzymes sequentially attaching to an XNA strand to produce an amino acid sequence utilizing up to eight amino acids.

Table I. Process of Protein and Gene Synthesis in a Protobiont

1. An XNA strand-like gene of about 300 A or U nucleobases, binary coded in triplets duplicates itself by template action with the help of XNA replicase, an enzyme catalyst.

2. Amino acyl synthetases with their corresponding amino acids would attach to and detach from the XNA strand in a consecutive, sequential manner (linear chain propagation rather than template action). Synthetases would have complementary recognition sites so they could link up sideways with each other when attaching to the XNA strand. With each visitation a single amino acid would be polymerized to the end of the growing polypeptide chain with the help of a protein polymerase enzyme.

3. When the end of the XNA strand was reached, the last synthetase would detach and the polypeptide chain would be set free. It would then fold into an enzyme molecule.

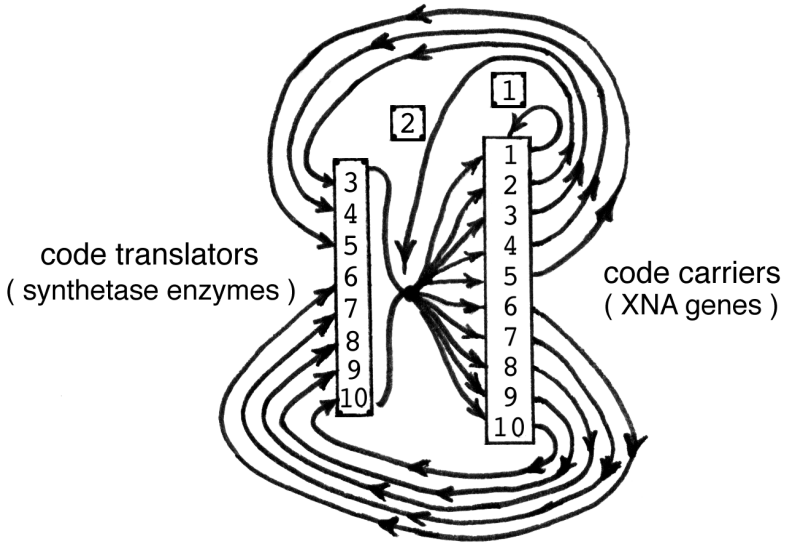

Fig. 3. Self-assembling, self-reproducing gene-protein catalytic cycle. Note that each gene would chain together many nucleobase triplets to code for a particular enzyme or nucleobase.

to decode all 10 genes to produce all 10 protein enzymes. In this instance, a self-assembling, self-reproducing catalytic cycle could have formed similar to that shown in Fig. 3. The left box denotes the eight code translators and the right box the 10 genes serving as the code carriers. Gene 1 would code the synthesis of the replicase enzyme [1] and gene 2 would code the synthesis of the polymerase enzyme [2].

Note that the individual enzyme or gene components serve as inputs to the total gene-enzyme system, and that the total system produces as its output more of these enzymes and genes. This autopoietic process may be understood in terms of system structure/function concepts and the interrelationship of micro and macro system hierarchic level. That is, in a self-perpetuating system such as this, an initial set of macromolecules, structures preexisting at the subsystem level, would produce a set of functions which at the system level would complement one another to produce a functionally ordered pattern (a metabolic network, or metabolic "mind") which in turn would be capable of reproducing these subsystem structures; see Fig. 4.

The chicken-egg problem is overcome by postulating that the initial protobiont (the initial X

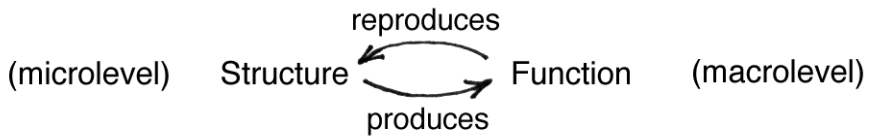

Fig. 4. Structure/function relationship in a protobiont.

concentration, X_0) emerged as the result of a fortuitous event in the aggregation of macromolecules. The chances of such an event occurring may be roughly estimated using an information theory technique suggested by R. W. Kaplan (1971, p. 319). Using his approach we may assign a probability of 10^{-8} (1 in 100 million) that a given amino acid sequence would yield the proper enzyme activity. This is based on the assumption that the amino acid chain length is approximately 100 monomers and that many of these monomers may be interchanged without significantly reducing the specific catalytic effect of the enzyme. Using the same information theoretic assumptions, we may assign a similar probability of 10^{-8} that a given 300 nucleobase gene sequence would yield the appropriate amino acid sequence when decoded in triplets. Also, if we assume that nucleobases are produced 10^{-6} times as frequently as polypeptides under abiotic conditions, we may adjust the product of these probabilities such that the total probability for emergence of a gene-enzyme pair comes out to be 10^{-22}. If we assume that the basic set consists of 20 polymers (10 genes and 10 proteins) and that this set must aggregate within a 20 μ diameter cellular microsystem. Using Kaplan's method, a probability of 10^{-8} is calculated that a basic set would be contained within a microsystem of this size. This means that for every gram of prebiotic proteinoid material (i.e., for every 100 million microspheres) one protobiont would be expected to emerge. Considering the fact that during this formative period the earth could have been covered with over 10^{16} grams of proteinoid material at a given time, the emergence of life-like microsystems appears to have been a highly probable event.

The various microsystem combinations of proteins with other proteins, and in some cases, of proteins with nucleobases could be regarded as combinatorial or *informational fluctuations* since each macromolecule would embody information in its structure. Variations in the pH of individual proteinoid microphere would have been another factor influencing the character of a given combinatorial set. When eventually a basic set of proteins and genes arose that had autocatalytic, self-producing properties, this event would have constituted a *critical fluctuation*. Once this particular set of macromolecules had been fortuitously brought together within a particular cellular microsystem (or within a community of conjugally associating microspheres), a new regime would have been entered, one of exponentially increasing informational coherence (order) rather than informational randomness. Thus, the principle of order-through-fluctuation, first suggested to describe the emergence of space/time order in nonlinear chemical reaction systems (Prigogine, Nicolis, and Babloyantz, 1972), is also applicable to the initial emergence of protobionts.

The catalytic cycle shown in Fig. 3 would be capable of reproducing XNA genes and catalyzing enzymes in a nonlinear manner. The overall behavior of such a system could be modeled by the autocatalytic reaction: $A + X \rightarrow 2X$, where A represents the amount of food (i.e., monomer building blocks and energy-releasing polyphosphates) and where X represents the amount of protobiont biomass, i.e., self-assembling gene/enzyme systems. Raw materials consumed within the microsphere would continually diffuse in from the environment. Thus, proteinoid microspheres would be open, not only to the transport of polymers (accretion), but

now also to the transport of monomers and inorganic phosphates (diffusion). If the self-assembly rate of the basic set is sufficiently rapid such that it is greater than the rates of polymer hydrolysis, then this microsystem will grow. And, if this rate of growth is sufficiently large compared to the rate of microsystem dissociation, this microsystem will fission and proliferate.

Eigen (1971) and Eigen and Schuster (1977, 1978a, 1978b) have elaborated a general theory to describe biological self-organization which is founded on the notion of the catalytic hypercycle. One class of hypercycles refers to chemical reaction sequences that self-close into self-reinforcing loops, while another class involves a set of gene-enzyme replication pathways that self-close to synthesize their constituents in autopoietic fashion. The scheme proposed in the present paper would be a version of this second hypercycle class whose most basic type is represented as $X \xrightarrow{I}$ I where X symbolizes component molecules and I sumbolizes an information carrying entity (Eigen and Schuster (1977, p. 544).

Eigen and Schuster (1978b, Fig. 45, pp. 341-342) have proposed a simpler basic gene-enzyme set than that presented here. Their self-replicator utilizes four translator enzymes, rather than eight, and does not code the synthesis of a polymerase enzyme. Thus their system requires a total of five enzymes, four translator enzymes (synthetases) and one replicase enzyme, and five gene strands. Also, their model assumes gene strands composed of RNA rather than XNA. Their utilization of four different codons in their model would imply that their codons use two different nucleobases organized into pairs, rather into triplets, e.g., AA, AU, UA, UU.

However, they found that the functioning of their five-gene replicator system deteriorated due to internal competition between the genes for the replicator enzyme. Simulations of a differential equation form of their model showed that, as time passed, one translator gene became dominant, being fabricated in increasing abundance at the expense of the others. The system eventually died as the rate of fabrication of the replicator enzyme approached zero due to insufficient amounts of the other translator enzymes. Consequently, they discarded it as a viable protobiant candidate and concluded that an effective gene-enzyme self-replicator would require the inclusion of additional reaction hypercycles to establish mutual control of the gene populations.

The gene-enzyme self-replicator, proposed here (Fig. 3) which has 10 genes in its basic set, however, should avoid this problem. One reason is that by having a greater number of genes, 10 instead of 5, competing for the common resource of replicator enzyme, it necessarily has greater stability. That is, any given gene in the 10-gene system would have half as much access to replicator enzyme and hence if its population were edging toward dominance, it would take much longer before achieving monopolist position. Another factor, one not addressed by Eigen and Schuster, is the effect of heterogeneous distribution. In the model proposed here, the basic set is contained within a microsphere (proteinoid or lipid), which has the effect of isolating the self-replicating gene-enzyme set within a distinct domain. If the parent microsphere with its self-replicating basic set grows and divides to yield a population of two or more generations of individually evolving microspheres, then there is greater survivability for the whole population of replicators. For example, if a basic set replicator in one microsphere evolved toward a death state, it would not impact the evolution of the replication processes occurring in the other microspheres. Fusion of microspheres could also counteract this competition effect. That is, if one microsphere contained a more balanced enzyme population and viable replication activity while the other had no replication activity due to depletion of certain necessary enzymes, the fusion of these two systems would average the enzyme concentrations and hence create conditions sufficient to allow continued reproductive activity.

The replicative activity of an interacting microsphere population would be characterized by a competitive interplay between the tendency for disproportionate growth of individual enzymes and the spatial diffusion of the enzyme products through microsphere division and fusion. This would resemble the competitive interplay between reaction and diffusion processes that operate in a nonlinear reaction-diffusion system. So it should be possible to test this survivability hypothesis by rendering it in partial differential equation form, as is done for modeling reaction-diffusion systems, and simulating the system. It may be that population-controlling reaction hypercycles are not required to ensure survivability. Stability would occur naturally provided that the self-reproducing basic sets were distributed among a sufficiently large population of interacting microspheres.

5. A new category of nonequilibrium system

There are many similarities between biological organisms such as living cells and dissipative structures such as those found in chemical reaction-diffusion systems. In both cases spatial and temporal ordering arises as a result of entropy dissipation associated with nonequilibrium processes constituting the system, and in both cases such ordering only arises when the system is maintained past a critical distance from equilibrium. However, it would be incorrect to seek to formulate a theory of the initial emergence of life solely on the basis of such chemical kinetic models. A careful analysis of the hypothetical gene-protein catalytic/autopoietic cycle discussed in the last section reveals that its overall character is different in a number of respects from that of chemical reaction systems such as the Belousov-Zhabotinskii reaction, the glycolytic cycle, or theoretical systems such as the Brusselator. For example, in such chemical reaction-diffusion systems, "order" arises as a consequence of spatiotemporal variations in the *concentrations* of various chemical species. This would be a pattern type of order, i.e., order associated with a reactant's concentration at different locations, or positions, in space or time. In such systems the values of the kinetic constants, diffusion coefficients, and chemical concentrations play an important role in determining the character of the emergent order. On the other hand, the type of ordering which emerges in a gene-protein cycle would be of functional character rather than positional. That is, the total amount of polymerized biomass (total amount of functional order) is the main point of interest here, not whether this biomass happens to be concentrated in a small dense cellular region or diluted over a very large area. Moreover, unlike reaction-diffusion systems, changes in kinetic constants, diffusion coefficients and reactant concentrations do not determine the kind of polymer order that emerges, only its rate of synthesis. In such gene-protein cycles the kind of ordering which takes place is determined primarily by the recipe embodied in the gene structure. So, based on these observations, it may be useful to refer to such gene-protein cycles by the classificatory term *synthetic nonequilibrium systems* to distinguish them from reaction-diffusion type nonequilibrium systems.

A protobiont (synthetic nonequilibrium system) should be distinguished from its immediate environment, i.e., the microsphere which surrounds it and which, besides the basic set, also contains non basic set polymers, monomers, and polyphosphates. Reaction processes by which the replicase and polymerase enzymes become activated by the energy-rich polyphosphates, although a vital part of the protobiont's metabolism, are not directly involved in the protein synthesis or genetic replication operations. Hence, they will be regarded as "external functions", and will be referred to as transmutational processes to distinguish them from gene-related synthetic processes. That is, they would constitute processes by which individual molecules (or macromolecules) would become chemically changed as a result of non-code-related reactions. Of

course, given enough time, the protobionts would evolve to the point where transmutational processes such as these would become included within the sphere of genetic control. At such a point it would be difficult to clearly separate the two types of processes. Regarding the more advanced living systems observed around us today, it must be admitted that they are made up of a complex intertwining of both synthetic and transmutational processes.

6. A thermodynamic analysis

The protobiont, being identified with the protein synthetic and gene replication operations, may be distinguished as a subsystem of the microsphere host cell. Both system and subsystem may be regarded as open systems engaged in processes of entropy change and exchange, as shown in Fig. 5. These processes may be understood as follows.

The increase in order taking place in the self-production process may be expressed thermodynamically by the equation:

$$\partial S/\partial t = \partial_a S/\partial t + \partial_c S/\partial t, \tag{1}$$

which is modeled after the Bertalanffy growth equation (Bertalanffy, 1968, pp. 171-184). The term, $\partial_a S/\partial t$, is the anabolic entropy change of the basic set, or in other words, the rate of entropy change associated with gene-related macromolecular assembly. This term is always negative ($\partial_a S/\partial t < 0$) since the polymerization of monomers is an order increasing process. The term, $\partial_c S/\partial t$, is the catabolic entropy production of the basic set, or the rate of entropy change associated with the dissociation of genetically assembled macromolecules. This term is always positive ($\partial_c S/\partial t \geq 0$) since dissociation is a disordering process. Now, if anabolism exceeds catabolism, $| \partial_a S/\partial t | > | \partial_c S/\partial t |$, then the net entropy will decrease ($\partial S/\partial t < 0$) and the system will grow and become more ordered. If catabolism exceeds anabolism, $| \partial_a S/\partial t | < | \partial_c S/\partial t |$, then

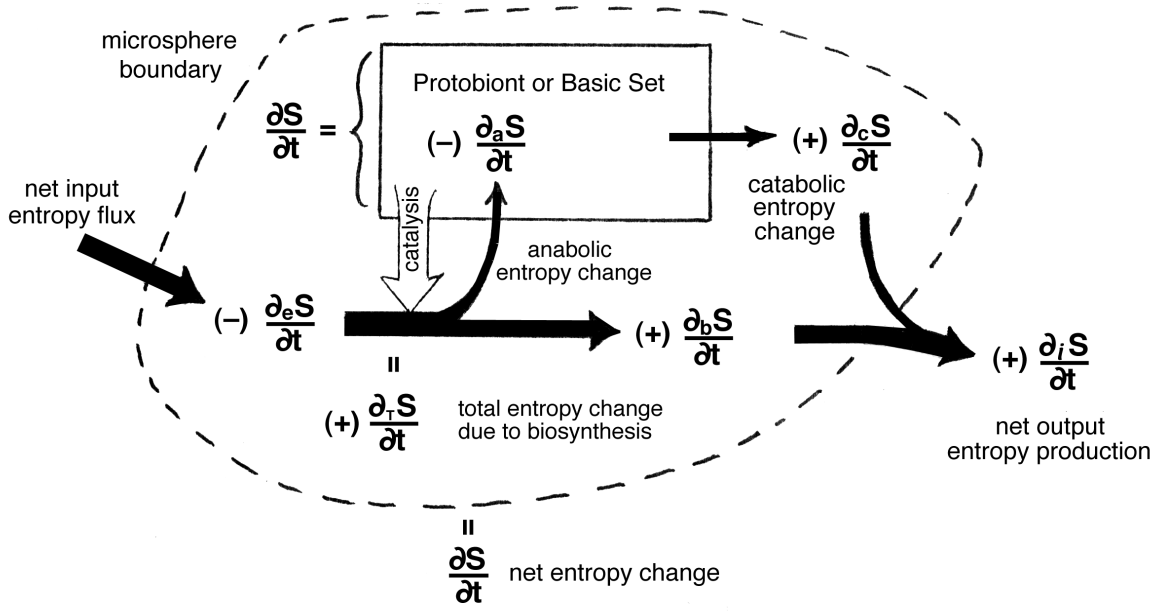

Fig. 5. Entropy change and exchange associated with a protobiont developing within a microsphere host.

the net entropy will increase ($\partial S/\partial t > 0$) and the system will atrophy and eventually disintegrate. Finally, if both are equal to one another, $| \partial_a S/\partial t | = | \partial_c S/\partial t |$, then the system will be in a steady state maintaining its state of order (or entropy) at a constant value. These processes are illustrated in the protobiont box in Fig. 5.

The anabolic entropy change, $\partial_a S/\partial t$, may be negative without violation of the Second Law of Thermodynamics which applies only to the total entropy change. The total entropy change is given by the relation:

$$\partial_T S/\partial t = \partial_a S/\partial t + \partial_b S/\partial t, \tag{2}$$

where $\partial_a S/\partial t < 0$, the entropy decrease of the basic set (the system), is outweighed by the larger quantity, $\partial_b S/\partial t$, the rate of entropy output to the environment, i.e., $| \partial_a S/\partial t | < | \partial_b S/\partial t |$. Consequently, $\partial_T S/\partial t > 0$. Note also that the rate of total entropy production, $P = \partial_i S/\partial t$, is the sum:

$$\partial_i S/\partial t = \partial_b S/\partial t + \partial_c S/\partial t, \tag{3}$$

which includes the energy and entropy dissipation created both by the system's anabolic and catabolic processes; see Fig. 5, right side of microsphere. Consequently, increased rates of growth, i.e., increased negentropy fixation or polymerization, necessitate increased rates of overall dissipation.

The net entropy balance for the microsphere as a whole would be given by the Prigogine Equation (Prigogine, 1945):

$$dS = d_e S + d_i S. \tag{4}$$

This relation considers the net entropy change dS during a time interval dt, where $d_e S$ is the entropy flux due to exchanges of energy or matter with the environment (i.e., import of energy-rich polyphosphates) and where $d_i S$ is the entropy production due to irreversible processes inside the system (i.e., diffusion, heat conduction, and chemical reactions). If $d_e S$ is negative and if $|d_e S| > |d_i S|$, then dS may also be negative without violation of the Second Law.

Equation (4) expressed in its partial differential form would be written as:

$$\partial S/\partial t = \partial_e S/\partial t + \partial_i S/\partial t. \tag{5}$$

This relation is essentially the same in content as equation (1). For example, adding the quantities $- \partial_b S/\partial t$ and $+ \partial_b S/\partial t$ to the right hand side of (1) yields:

$$\partial S/\partial t = [\partial_a S/\partial t - \partial_b S/\partial t] + [\partial_c S/\partial t + \partial_b S/\partial t] \tag{6}$$

Moreover since $\partial_a S/\partial t < 0$ here, using relation (2) to substitute for $\partial_a S/\partial t$ in the first term and relation (3) to substitute for $\partial_c S/\partial t$ in the second term, this converts to:

$$\partial S/\partial t = -\partial_T S/\partial t + \partial_i S/\partial t + \partial_b S/\partial t. \tag{7}$$

Since $\partial_e S/\partial t = -\partial_T S/\partial t$, equation (7) is similar to (5) with the exception that (5) does not include the intermediate positive entropy change term $\partial_b S/\partial t$. So, the only difference between equations (1) and (5) is whether or not they take into consideration the intermediate term $\partial_b S/\partial t$ which represents the entropy production due to biosynthesis.

7. The evolution of life

Once a protobiont emerged in the form of a basic set, incorporating functions of coding, translation, polymerization and replication, it was vulnerable to mutation through the action of various mutagens. Parasitic genes could have arisen making use of the reproductive enzyme, replicase, produced by the basic set. Such mutant strains would have placed a burden on the

basic set apparatus slowing down its rate of reproduction and placing that particular cellular microsystem at an evolutionary disadvantage relative to healthier systems. Only when a mutant arose which was able to synthesize an enzyme capable of catalyzing more rapid reproduction of the basic set, would there have been favorable selection. Thus, in the early heterotrophic stage, biological evolution would have proceeded through a series of transitions toward greater complexity and increased rates of dissipation. That is, the entropy production, $P = \partial_i S/\partial t$, would have increased over time, $\partial P/\partial t > 0$.

A "bootstrap" process similar to the evolutionary feedback scheme proposed by the Brussels school (Prigogine, Nicolis, and Babloyantz, 1972; Nicolis and Prigogine, 1977) is shown in Fig. 6. This may be understood as follows. It is assumed that there is a protobiont species X_1 which exists in the presence of food (the nonequilibrium condition). As the population of species X_1 increases, the probability that this population will develop a mutation also increases. A genetic mutation may be thought of as a "structural fluctuation," that is, a perturbation in the gene structure. Now, suppose that one of the genes in an X_1 cell mutates. If this fluctuation is a parasitic mutation, it will lead to a decreased rate of dissipation for the cell as a whole, i.e., a decreased growth rate relative to the other cells. Hence, such a fluctuation will be evolutionarily self-extinguishing. The population of X_1 protobionts will, therefore, remain unaffected, i.e., the gene pool will remain stable with respect to this parasitic fluctuation. On the other hand, a favorable mutation would increase the protobiont's rate of synthesis and dissipation giving it a competitive edge. Since the presence of this mutation would serve to enhance the rate of cellular biosynthesis relative to X_1 cells, under the prevailing nonequilibrium conditions, this fluctuation would become amplified. That is, it would constitute an *instability*; see Fig. 6.

Eventually, due to their enhanced rate of replication, the mutant cells would tend to proliferate and would come to constitute a new community of protobionts X_2 having an increased rate of dissipation relative to species X_1. If it is assumed that both reference species X_1 and the mutant species X_2 occupy the same ecological niche, then as a result of competition, the population of X_1 would decline to extinction and the population of X_2 would rise as shown in Fig. 7 (see Nicolis and Prigogine, 1977, p. 457; Prigogine, Allen, and Herman, 1977, p. 48-53). Species X_2 may be viewed from an evolutionary perspective as a macroscopic fluctuation in genetic composition that has become stabilized. As the favorable mutation X_2 becomes more prevalent, the probability increases that this mutant community will itself spawn a successful mutation, i.e., develop an unstable fluctuation. With a favorable fluctuation the cycle is repeated, i.e., a new mutant specie, X_3 develops having a higher rate of synthesis and dissipation and gradually succeeds species X_2. Thus, under conditions of competition, evolution proceeds through a series of metastable states to increasing levels of dissipation. We may, therefore conclude that dissipation is the *driving force* of biological evolution (Nicolis and Prigogine, 1977,

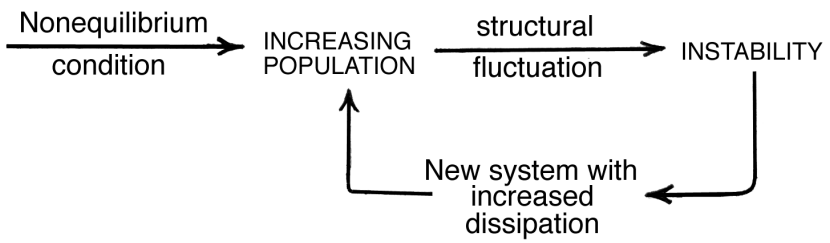

Fig. 6. A model of evolutionary feedback.

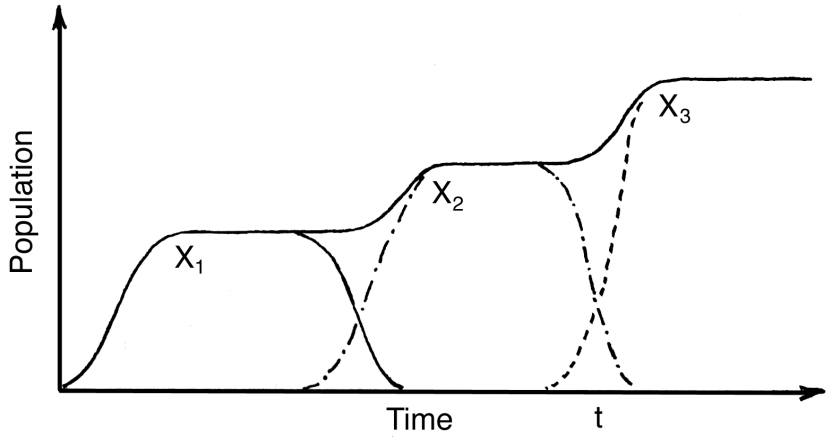

Fig. 7. An ecological niche occupied successively by species of increasing effectiveness.

p. 442).*

Mutations may also lead to the development of increasing varieties of species in cases where the mutant species explores a new ecological niche. To the extent that its niche is isolated from competition with other species, the level of dissipation that a mutant species exhibits relative to other species becomes less important. Hence, numerous varieties of species having different levels of dissipation may coexist simultaneously. Thus, biological evolution proceeds towards states of both increasing diversity and increasing dissipation.

The radiation of species into ecological niches may be compared to the manner in which gas molecules confined to a small space will tend toward a more probable distribution when allowed to expand into an open space. Thus, it is not surprising to find a close similarity between the Second Law of Thermodynamics and biological evolution.* Indeed, as Nicholas Georgescu-Roegen notes (1971, p. 130), in 1865 when R. Clausius formulated the Second Law, he coined the term "entropy" from a Greek word equivalent in meaning to "evolution". In making a comparison between the evolution of molecular states in a gas and the evolution of genetic states in a gene pool, molecular collision may be considered analogous to a genetic mutation and the resulting molecular state (position, velocity, direction) may be considered analogous to the resulting genetic state (nucleobase sequence). Whereas the spectrum of states available to an evolving mechanical system are limited by the laws of collision (conservation of energy and momentum), the spectrum of states available to an evolving living system are limited by the requirement that mutations must be favorable, i.e., they must ensure the survival of the mutant species in the context of its physical and biological environment.

8. Concluding Remarks

The symbiotic relationship between the basic set replicator and its enclosing microsphere, described in Section 4, with sufficient evolution could have led to the development of early

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* It is interesting to note that mutant species appear to surface as adaptive alternatives in times of stress. For example, If mutations appear in a reference population undergoing exponential growth, they will generally maintain a low profile, even though they may have a higher rate of synthesis. However, if the growth rate of the reference population begins to slacken due to environmental changes such as depleting food resources, mutant species possessing a growth rate advantage can more readily overtake their established competition.

bacteria utilizing a more contemporary RNA utilizing four-base codons. As the evolution of life proceeded, protobionts would have gradually replaced their proteinoid or lipid cellular enclosures, which nature had graciously provided for them, with their own synthetically produced cell membranes and internal structures. The genetic code would have become expanded to the contemporary 4 digit triple code, specifying instructions for polymerizing as many as 20 biologically produced amino acids. Code-related protein synthesis would eventually have been conducted by "indirect translation" utilizing messenger RNA, primitive ribosomes, transfer RNA, and synthetases) and the use of DNA would have become specialized for long-term information storage.

Cells at this primitive stage of development would have had few internal organelles, and genetic material would have been dispersed throughout the cell cytoplasm. These primitive prokaryotes would have been heterotrophic, feeding on amino acids in their environment. They would have derived their free energy from the oxidative phosphorilation of geophysically produced polymers or could have utilized simple photochemical reactions as an energy source. Primitive organisms of this variety could have existed as early as 4 billion years ago. By this time, the atmosphere could have become oxidized (through the photolysis of water) to one containing primarily nitrogen with some carbon dioxide and water vapor.

Further evolution of these prokaryotes would have produced the anaerobic heterotrophic bacteria and later the anaerobic photosynthetic autotrophic bacteria capable of synthesizing organic substances directly from dissolved carbon dioxide and nutrients (Margulis, L., 1971, p. 481). This branch of bacteria would have led to the evolution of oxygen tolerant, oxygen producing blue-green algae, which are believed to have been present as early as 3.4 billion years ago according to recent fossil discoveries of Elso Barghoorn. The oxygen released by these photosynthesizing bacteria would have led to a gradual increase in the level of atmospheric oxygen and to the formation of an ozone layer thick enough to shield the earth from harmful UV radiation. This shielding would have allowed life to inhabit previously uninhabitable regions. With the increased oxygen content in the atmosphere, oxygen respiring aerobic bacteria would have evolved including "protomitochondria" (Margulis, 1971). By this time the terrestrial, aerial, freshwater, marine and hot springs environments would have literally teemed with bacteria.

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References

Baltsheffsky, H., 1971, Inorganic pyrophosphate and the origin and evolution of biological energy transformation, In: C. Ponnamparuma and R. Buvet, eds.) *Molecular Evolution I* (New York: North-Holland/ American Elsevier).

Bertalanffy, L. von, 1968, *General System Theory* (New York: George Braziller).

Budin, I., Debnath, A., Szostak, J. W., 2012, Concentration-driven growth of model protocell membranes, *J. American Chemical Society* **134**, 20812-20819.

Callahan, M. P., et al., 2011, Carbonaceous meteorites contain a wide range of extraterrestrial nucleobases, *Proceedings of the National Academy of Sciences* **108**, 13995-13998.

Deamer D. W., 1985, Boundary structures are formed by organic components of the Murchison carbonaceous chondrite, *Nature* **317** (6040):792-794.

Deamer D. W., 1986, Role of amphiphilic compounds in the evolution of membrane structure on the early earth, *Origins of Life and Evolution of the Biosphere*, **17** (1):3-25.

Dubina, M., et al., 2013, Potassium ions are more effective than sodium ions in salt induced peptide formation, *Orig Life Evol Biosph.* **43**, 109-117.

Eigen, M., 1971, Self-organization of matter and the evolution of biological macromolecules, *Die Naturwissenschaften* **58**, 465-523.

Eigen, M., Schuster, P., 1977, The hypercycle: A principle of natural self-organization. Part A. Emergence of the hypercycle, *Naturwissenschaften* **64**, 541-565.

Eigen, M., Schuster, P., 1978a, The hypercycle: A principle of natural self-organization. Part B. The abstract hypercycle, *Naturwissenschaften* **64**, 7-41.

Eigen, M., Schuster, P., 1978b, The hypercycle: A principle of natural self-organization. Part C. The realistic hypercycle, *Naturwissenschaften* **64**, 341-369.

Fox, J. L., 1972, Origins, In: D. Rohlfing and A. Oparin (eds.), *Molecular Evolution* (New York: Plenum Press).

Fox, R. F., 1972, A Non-equilibrium thermodynamical analysis of the origin of life, In: D. Rohlfing and A. Oparin (eds.), *Molecular Evolution* (New York: Plenum Press).

Fox, S. W., 1971, Self-assembly of the protocell from a self-ordered polymer, In: A. Kimball and J. Oro (eds.) *Prebiotic and Biochemical Evolution* (New York: North-Holland/American Elsevier).

Fox, S. W., 1971, Chemical Origins of Cells--2, *Chemical and Eng. News* **49**, 46-53.

Georgescu-Roegen, N., 1971, The entropy law and the economic process (Cambridge, MA: Harvard Huniversity Press).

Hochstim, A., 1971, The role of shock waves in the formation of organic compounds in the primeval atmosphere, In: C. Ponnamperuma and R. Buvet (eds.), *Molecular Evolution I* (New York: North-Holland/American Elsevier).

Hsu, L., 1972, Conjugation of proteinoid microspheres: A model of primordial recombination, In: D. Rohlfing and A. Oparin (eds.), *Molecular Evolution* (New York: Plenum Press).

Jungck, J., 1972, Thermodynamics of self-assembly: An empirical example relating entropy and evolution, In: D. Rohlfing and A. Oparin (eds.), *Molecular Evolution* (New York: Plenum Press).

Kaplan, R. W., 1971, The problem of chance in formation of protobionts by random aggregation of macromolecules, In: C. Ponnamparuma and R. Buvet (eds.), *Molecular Evolution I* (New York: North-Holland/ American Elsevier).

Kulaev, I., 1971, Inorganic polyphosphates in evolution of phosphorus metabolism, In: C. Ponnamparuma and R. Buvet (eds.), *Molecular Evolution I* (New York: North-Holland/American Elsevier).

Kulaev, I., 1974, The role of inorganic polyphosphates in chemical and biological evolution, In: K. Dose, S. Fox, G. Deborin, and T. Pavlovskaya (eds.), *The Origin of Life and Evolutionary Biochemistry* (New York: Plenum Press).

Margulis, L., 1971, Microbial evolution on the early earth, In: C. Ponamparuma and R. Buvet

(eds.), *Molecular Evolution I* (New York: North-Holland/American Elsevier), pp. 480–484.

Madnikov, B., 1971, The origin of ribosomes and the evolution of rRNA, In: C. Ponnamperuma and R. Buvet (eds.), *Molecular Evolution I* (New York: North-Holland/American Elsevier).

Maturana, H. R. and F. G. Varela, 1973, De maquinas y seres vivos (Editorial Universitaria, Santiago).

Miller, S., and M. Parris, 1971, Synthesis of pyrophosphates under primitive earth conditions, In: A. Kimball and J. Oro (eds.), *Prebiotic and Biochemical Evolution* (New York: North-Holland/ American Elsevier).

Nakashima, T., Fox, S. W., 1980, Synthesis of peptides from amino acids and ATP with lysine-rich proteinoid. *J. Mol. Evol.* **15**, 161-168.

Nicolis, G. and I. Prigogine, 1977, *Self-organization in Nonequilibrium Systems* (New York: Wiley-Interscience).

Oparin, A., 1924, Proiskhozhdenie zhigni (Origins of life), *Izd. Moskovskii rabochii*, Moskva.

Plankensteiner, K., Reiner, H., & Rode, B. M., 2004, Prebiotic formation of amino acids and peptides and a possible origin of homochirality, In: Proceedings of the Third European Workshop on Exo-Astrobiology, 18 - 20 November 2003, Madrid, Spain. Ed.: R. A. Harris & L. Ouwehand. ESA SP-545, Noordwijk, Netherlands: ESA Publications Division, p. 263 - 264.

Plankensteiner K, Reiner H, Rode BM, 2006, Amino acids on the rampant primordial Earth: electric discharges and the hot salty ocean, *Molecular Diversity* **10**, 3-7.

Podkletnov, N. E., Markhinin, E. K., 1981, New data on abiogenic synthesis of prebiological compounds in volcanic processes, *Origins of Life* **11**(4), 303-15.

Prigogine, I., 1945, *Bull Acad. Roy. Belg. Cl. Sci.* **31**, 600-606.

Prigogine, I., G. Nicolis, and A. Babloyantz, 1972, Thermodynamics of evolution, *Physics Today*, **25** (11 & 12).

Prigogine, I., P. Allen, and R. Herman, 1977, The evolution of complexity and the laws of nature, In: E. Laszlo and J. Bierman (eds.), *Goals in a Global Community* (New York: Pergamon Press), pp. 48-53.

Rode, B. M., 2013, Peptides and the origin of life, *Peptides* **20**, 773-786.

Rohlfing, D. L., 1976, Thermal polyamino acids: synthesis at less than 100 degrees C, *Science* **193,** 68-70.

Saladino, R, et al., 2001, A possible prebiotic synthesis of purine, adenine, cytosine, and 4(3H)-pyrimidinone from formamide. Implications for the origin of life, *Bio org Med Chem* **9**: 1249–1253.

Schrum, J.S., Zhu, T.F., Szostak, J.W., 2010, The origins of cellular life. Cold Spring Harbor Perspectives in Biology (Cold Spring Harbor Laboratory Press).

Schwartz, A., 1972, The sources of phosphorus on the primitive earth--An inquiry, In: D. Rohlfing and A. Oparin (eds.), *Molecular Evolution* (New York: Plenum Press).

Stoks, P. G., Schwartz, A. W., 1979, Uracil in carbonaceous meteorites, *Nature* **282**, 709-710.

Stoks, P. G., Schwartz, A. W., 1981, Nitrogen-heterocyclic compounds in meteorites – Significance and mechanisms of formation, *Geochimica et Cosmochimica Acta*, **45**, 563-569.

Toupance, G., F. Raulin, R. Buvet, 1971, Primary transformation processes under the influence

of energy for models of primordial atmospheres in thermodynamic equilibrium, In: C. Ponnamperuma and R. Buvet (eds.) *Molecular Evolution I*,(New York: North-Holland/American Elsevier).

Varela, F. G., H. R. Maturana and R. Uribe, 1974, Autopoiesis: The organization of living systems, its characterization and a model, *BioSystem*s **5**, 187-196.

Zaleski, D. P., et al., 2013, Detection of E-cyanomethanimine towards Sagittarius B2(N) in the Green Bank Telescope PRIMOS Survey, *ApJ Let* **765**, L10.