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THE SEARCH FOR THE CHEMISTRY OF LIFE'S ORIGIN

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A central *postulate* of contemporary natural science states that life emerged on Earth (or elsewhere) through a transition of chemical matter from *non-living* to *living*. The transition is seen as a *contingent* consequence of the second law of thermodynamics and the chemical properties of matter by one group of scientists, and as an *imperative* of that law and those properties according to the belief of others. Chemical matter is postulated to have been capable of organizing itself out of disorder by channeling exergonic geochemical reactions into reaction networks that had a *dynamic structure* with kinetic (as opposed to thermodynamic) stability and were driven by autocatalytic molecular replication cycles. The postulate implicates that such chemical systems eventually became *self-sustaining* (capable of exploiting environmental sources for reconstituting itself), *adaptive* (capable of reacting to physical or chemical changes in the environment such that survival as a system is maintained) and – by operating in compartments – capable of *evolving*. From this perspective, *life's origin is seen as* a *seamless transition from self-ordering chemical reactions* to *self-sustaining chemical systems that are capable of Darwinian evolution* [1]. Figure 1 delineates – in terms of a 'conceptual cartoon' – such a programmatic view in more detail.

Evidence from paleontology, biology, geology and planetary science posits the appearance of life on Earth into a period of 3 to 4 billion years ago. Whereas the course of biological evolution is documented by a wealth of fossils of extinct organisms and, more recently, by information from comparative analysis of the genomes of biological species, there are no 'fossils' that would reliably document the nature of the molecules that were involved in the chemical processes at the dawn of life. We do not know whether at the beginning there existed a multitude of different life forms from one of which the one we know today has derived, neither do we know whether the type of molecules and chemical processes on which such

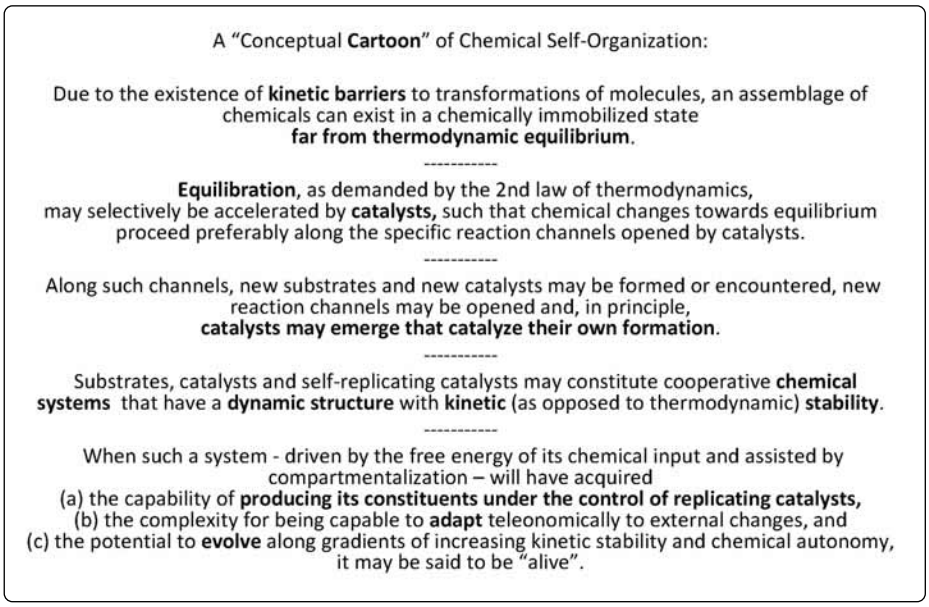


Figure 1. A 'conceptual cartoon' of chemical matter's self-organization towards life.

ancestral lives may have been based were or were not akin to the biological molecules and processes we are familiar with today. Such uncertainty notwithstanding, observations made in half a century of prebiotic chemistry (see below) point to a high probability for an origin of life scenario, in which the continuity postulated to have connected the emergence of adaptive behaviour on the chemical level with the beginnings of evolutionary processes on the biological level was paralleled by a constitutional continuity in the type of molecules that were involved in the transition. This continuity is supposed to be embodied in the chemical structures of α-amino acids, sugars, nucleobases, cofactor molecules and, in addition, in basic biochemical reactions that we find operating still today as enzyme-assisted processes in primitive anaerobic microbes.

The experimental search for the chemistry of life's origin has been proceeding under the label 'prebiotic chemistry' for more than half a century now. This field of research has its conceptual roots in the writings of the Russian biochemist *A.I. Oparin* [2] and the British biologist *J.B.S. Haldane* [3] who, around the first quarter of the last century, independently propounded for the first time explicit views on a natural chemical origin of life

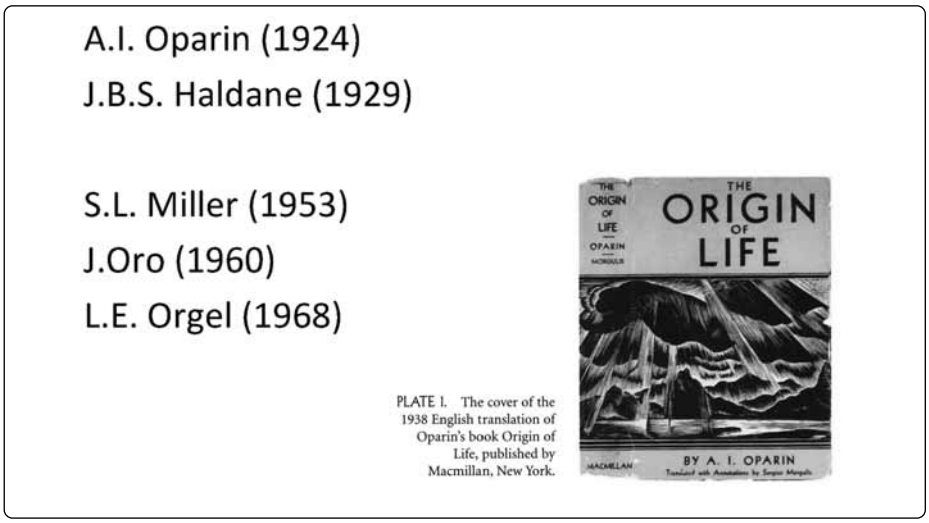


Figure 2. Pioneers of conceptual and experimental prebiotic chemistry in the last century [2][3][4][5].

on Earth (Figure 2). These views were launched into public awareness by the famous experiment of *Stanley L. Miller* [4] in 1953, where it was shown that hammering with excess of energy on gaseous mixtures of hydrogen, methane, ammonia and water induces the formation of – apart from large amounts of undefined organic material – the simplest representatives of the family of proteinogenic α-amino acids (Figure 3). In 1960, the Catalanian biochemist *Juan Oro* [5] discovered the formation of adenine – a molecule prototypical of contemporary biology – from HCN (hydrocyanic acid) in aqueous solution (Figure 4). *Leslie D. Orgel,* the last of the chemical pioneers listed in Figure 2, initiated in 1968 systematic experimental work towards the non-enzymic simulation of biology's arguably most important life process, the autocatalytic replication of nucleic acids [6].

Hydrocyanic acid (HCN), an unambiguously elementary, highly reactive organic molecule, is a central intermediate in Miller-type experiments [4,7] and known to be present on celestial bodies such as Titan and others (Figure 5), as well as to exist in astronomical quantities in interstellar space [8}. Chemically highly significant coincidences were observed between the constitutional spectrum of products formed in Miller-type experiments and the spectrum of organic compounds found in carbonaceous meteorites [9]. A recently published long-time/low-temperature experiment (Figure 6)

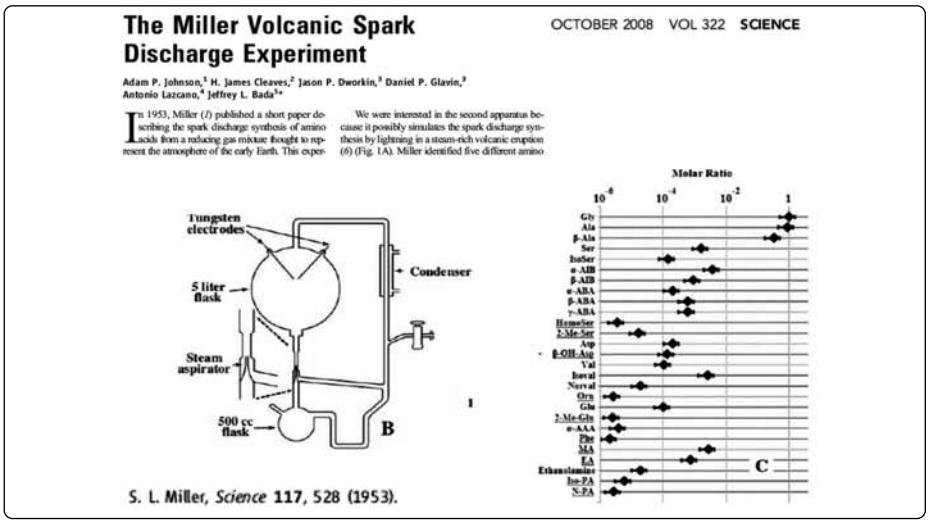


Figure 3. Recent re-analysis by modern analytical methods of the composition of authentic product mixtures obtained by *Stanley Miller* (deceased 2005) in the 1950s [7]. Absolute and relative amounts of biomolecules are still extremely low in such experiments, yet higher than observed before, and many more different molecules have now been identified.

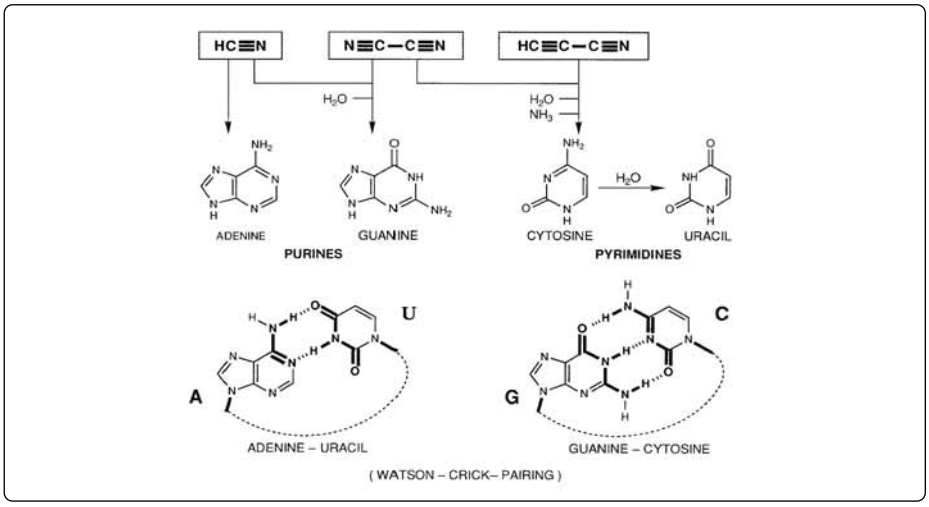


Figure 4. The central biomolecular structure of adenine is composed – formally as well as experimentally – of five molecules of HCN. A close and equally astonishing chemical relationship exists between related elementary carbon/nitrogen compounds and other canonical nucleobases. The lower part of the Figure depicts the two canonical Watson-Crick base-pairs, one of the, if not the, most fundamental biomolecular interactions in whole biology.

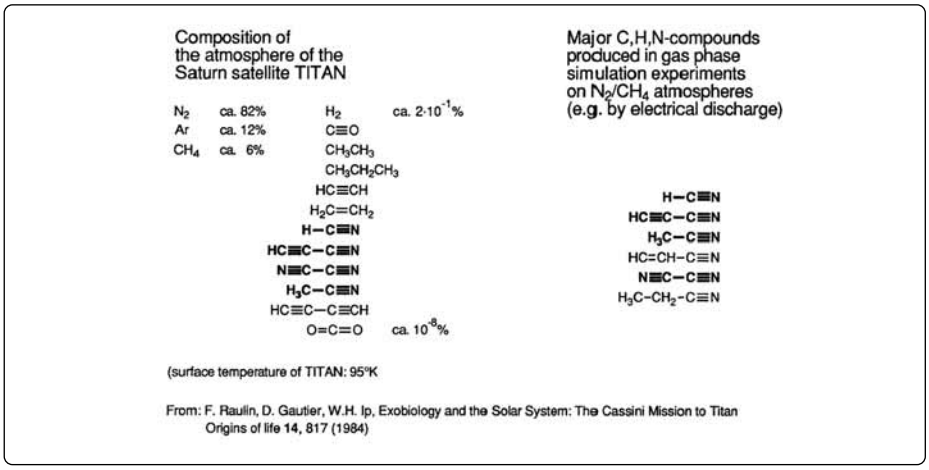


Figure 5. Whenever excessive energy hammers on carbon, nitrogen and hydrogen containing material of any sort, highly reactive carbon/nitrogen/hydrogen compounds such as HCN and higher derivatives of it (nitriles) are formed. Some of them (see formulae in bold, with triple-bonds) are highly reactive and, as HCN itself, chemically closely related to the structure of biomolecules. Note the similarity in the structure type of nitriles of extraterrestrial (natural) and terrestrial (experimental) origin.

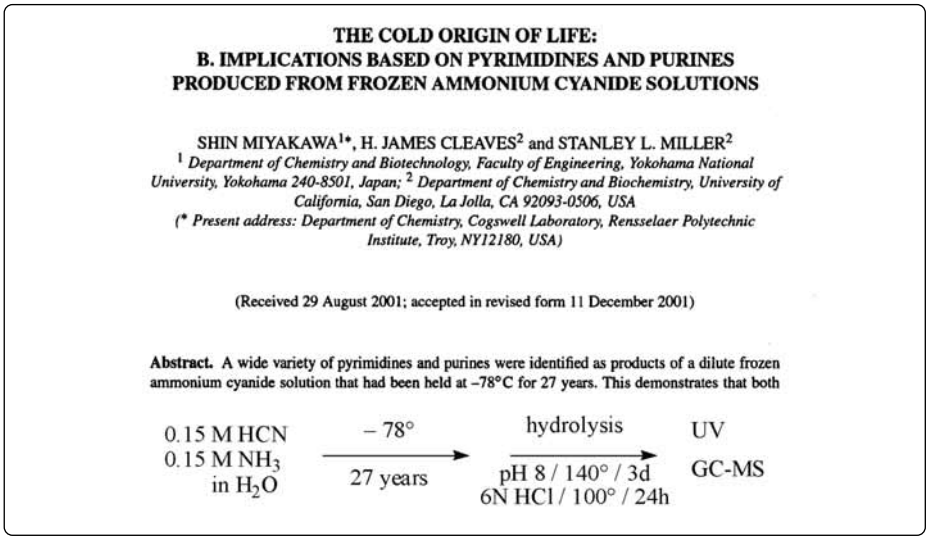


Figure 6. In a recently disclosed remarkable long-time/low-temperature experiment by *Stanley Miller* and (former) collaborators [10], the ammonium salt of HCN in aqueous medium was frozen to solid CO2 temperature, kept for 27 years, and finally its product composition analyzed after hydrolysis with aqueous acid or aqueous base (see Figure 7).

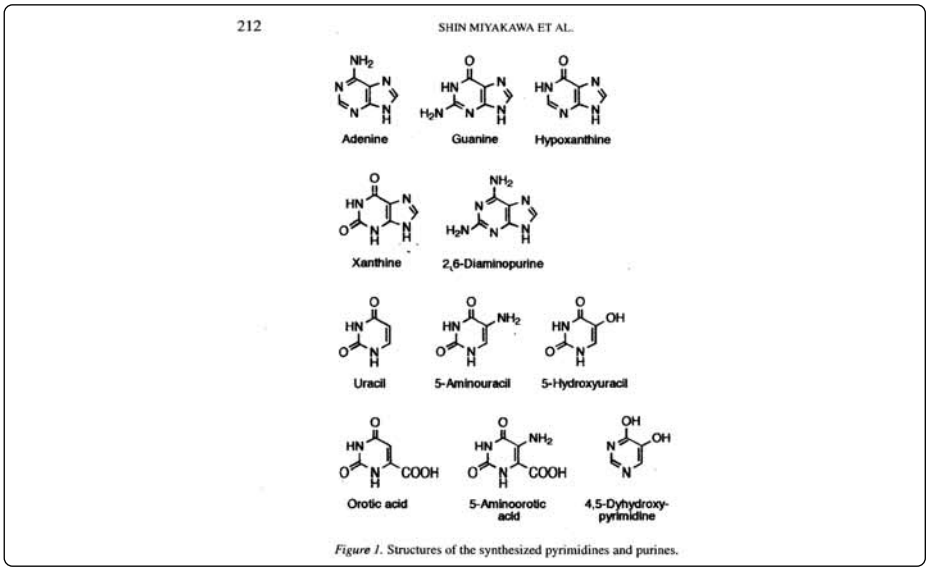


Figure 7. Chemical structures of heterocyclic organic compounds that have been identified (out of a mixture containing a large number of unidentified components) in the experiment described in Figure 6. Among the identified components are two canonical purines (adenine and guanine) and one canonical pyrimidine (uracil), besides two purines (hypoxanthine, xanthine) and one pyrimidine (orotic acid) that are part of the contemporary metabolism.

most impressively demonstrates the remarkably close chemical relationship between HCN and some of the fundamental biomolecules. Prominent in the palette of identified products of that experiment (Figure 7) are canonical purines and pyrimidines, basic constituents of the contemporary nucleic acids [10].

Experimental prebiotic chemistry suffers from the kind of handicap that is inherent in empirical research on historic processes. One is reminded of the fate of the anthropologist's Thor Heyerdahl famous Kon-Tiki experiment in 1947 (Figure 8) [11] by which it had been demonstrated that the original population of the Polynesian Islands could have come from South America. Yet that splendid demonstration of what is technically possible became eventually overridden by criteria anthropological in nature, convincing scientists that Polynesia's original population came from Asia.

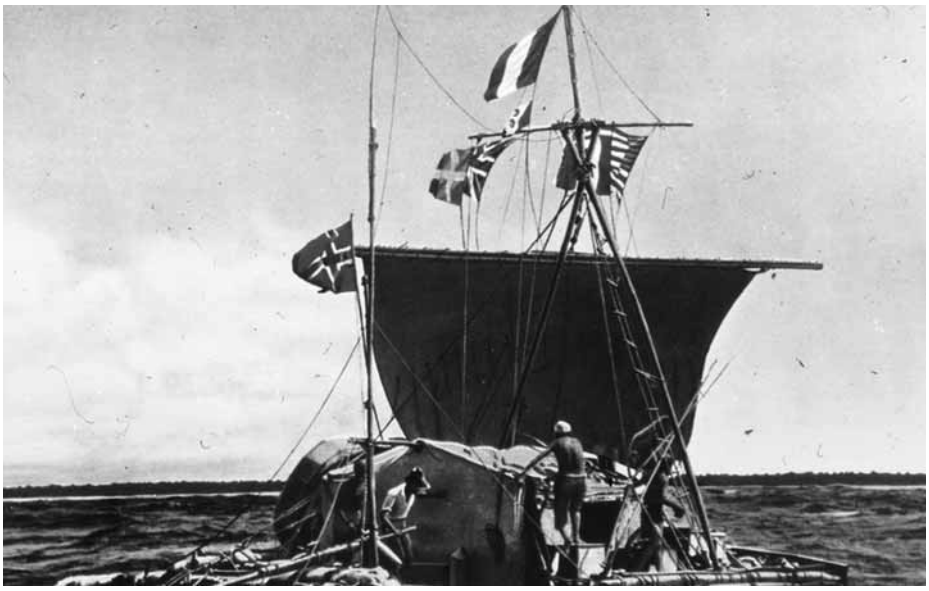


Figure 8. The balsa raft 'Kon-Tiki' on which the Norwegian anthropologist *Thor Heyerdahl* and a crew of five men embarked on a legendary expedition on April 28th 1947, starting in Callao (Peru). After 108 days they landed on the Polynesian Tuamotu Islands, thus demonstrating experimentally that Heyerdahl's theory, according to which the population of the Polynesian Islands came originally from South America, is compatible with what is technically feasible. Ironically, based on criteria anthropological in nature, scientists eventually convinced themselves that the Polynesian population originally came from Asia.

The possibility notwithstanding that conclusions in prebiotic chemistry eventually might suffer a fate similar to that of the Heyerdahl-experiment, the experimental results accumulated over the last half century [12] are in any case of lasting significance and importance. This is true irrespective of whether the organic material that had accumulated on the primordial Earth as the result of (geochemical) prebiotic processes and been delivered to the Earth by carbonaceous meteorites [9] was, or was not, relevant for life's actual emergence. What experimental prebiotic chemistry did achieve, is to conclusively demonstrate that the major types of low-molecular-weight building blocks of the life we know today have chemically *elementary* structures, elementary in the sense that their formation from (essentially) the chemical elements proceed quasi deterministically under an extraordinari-

ly broad range of (potentially geochemical) conditions (Figures 5 and 9). This does not necessarily mean, however, that those prebiotic organics of terrestrial or extraterrestrial origin in the primordial Earth were actually the starting materials for the critical self-organization process. In fact, there are two sharply opposing views on this point: the notions of a *heterotrophic* [2][4][12][13] versus an *autotrophic* [14][15] origin of life (Figure 10).

The proponents of a *heterotrophic* origin take for granted that the accumulation of organic matter by high energy processes on Earth, or by delivery to the Earth by meteorites, was the chemical source for the process of self-organization eventually leading to life's origin. In contrast, the concept of an *autotrophic* origin maintains that any such globally distributed mixture of organic material was *irrelevant* to the process(es) that led to self-organization. Reasons brought forward against heterotrophy refer to problems of selection, accumulation and concentration of specific substrates out of complex mixtures of chemicals, and of combinatorial reactivity and the short survival times of chemically activated substrates in unorganized chemical environments. The concept of autotrophy postulates the emergence of *de novo* pathways to starting materials and intermediates from elemental geochemical sources as an integral part of the very process that constituted self-organization (Figure 11). From the chemical viewpoint, both concepts are burdened with a great many open questions, such as the chemical nature of start-up substrates and catalysts, of primordial metabolism, of primordial replicating entities, be they metabolic or genetic cycles, the role and nature of compartmentalization, last but not least cellularization. Leaning towards one or the other of the two concepts remains still today a matter of reasoned opinion. This should not be taken as being scientifically contra-productive, since in any search for events of the past, commitment to basically different views leads committed researchers to focus on correspondingly different experimental strategies, that in turn may lead to potentially complementary insights.

Besides the debate on heterotrophy versus autotrophy, there is another dichotomy dividing researchers into two camps in their conceptual and experimental search for the chemistry of life's origin: the 'geneticists' [16-18], versus the 'metabolists' [14,15,21]. While both agree on the postulate that crucial to any beginning must have been the emergence of chemical reaction cycles that amounted to autocatalytic replication of molecules (Figure 11), the two camps differ in their view about the chemical nature of those cycles (Figure 12). The controversy [22,23] between the 'geneticists' and the 'metabolists' is the denial by the former of a claim made by the lat-

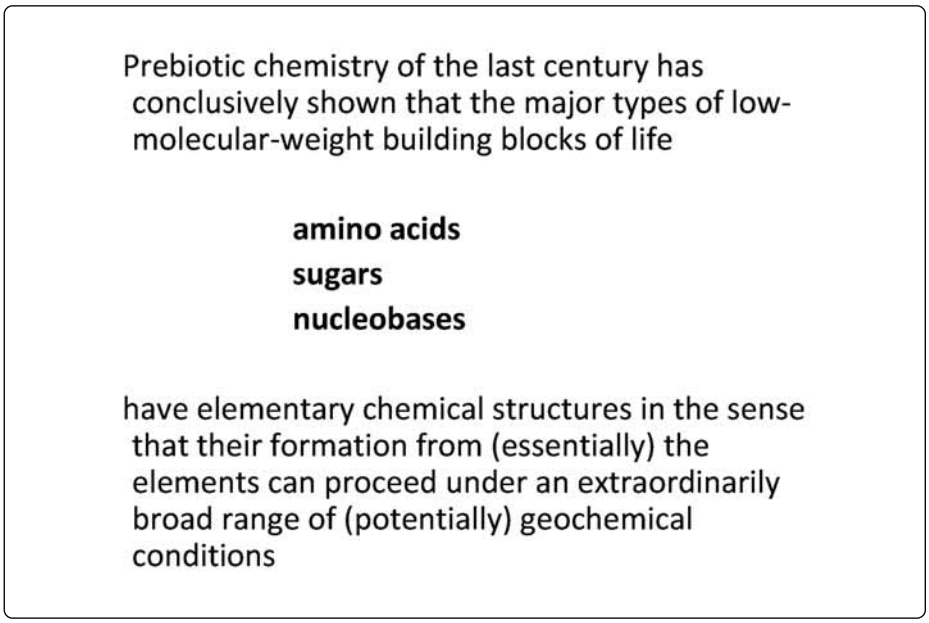


Figure 9.

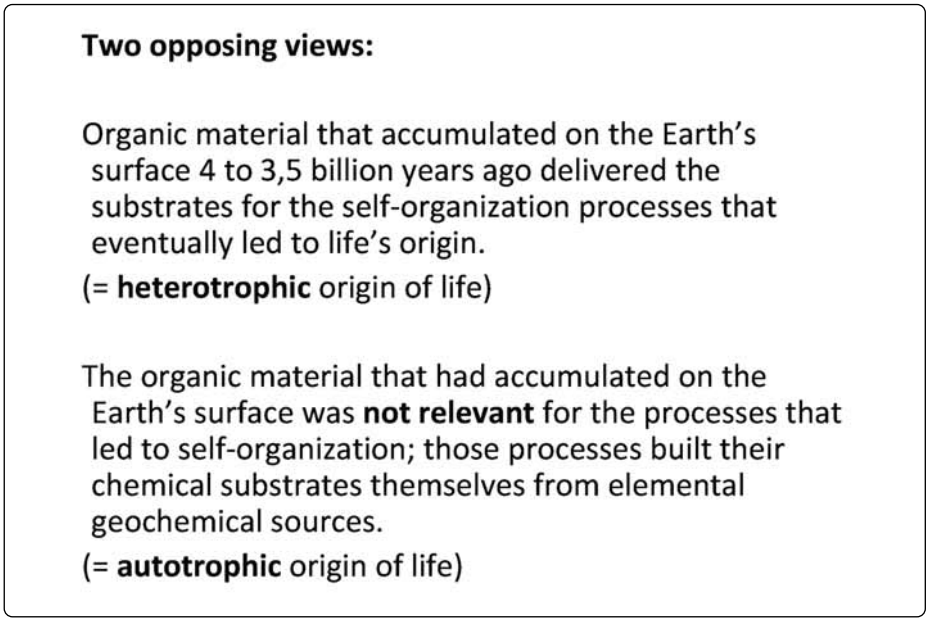


Figure 10.

ter, which is, that a chemical 'metabolic' system may have been capable to evolve and become 'alive' before it acquired a genetic system. To 'geneticists', the indispensible prerequisite for the emergence of a chemical system that deserves to be called 'alive' is the operation of a primordial genetic system. Geneticists challenge the view that autocatalytic metabolic cycles could have evolved with any degree of efficiency. They point to the paucity of such a type of cycles with regard to constitutional diversity and flexibility, as contrasted with replicating informational oligomers with their potential to store structural information in the form of a quasi unlimited constitutional diversity (sequence of specific recognition elements) and, therewith, the chance to give rise in principle to a large spectrum of phenotypic catalytic capabilities.

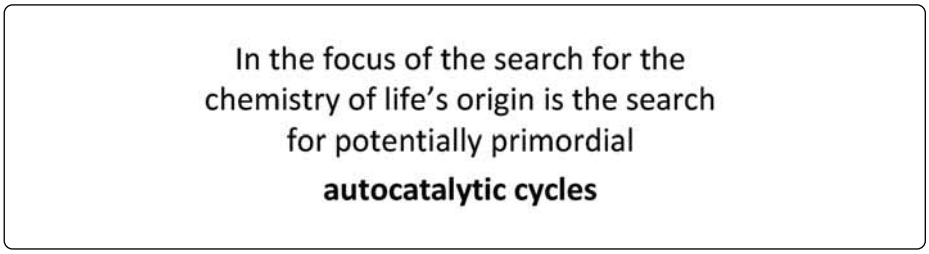


Figure 11.

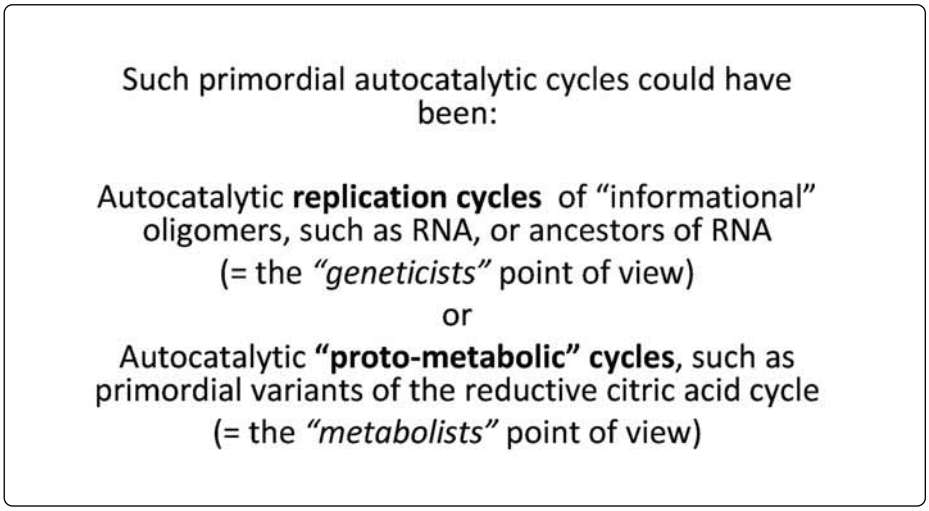


Figure 12.

What the standpoint 'metabolism first' in the debate between metabolists and geneticists implies is perhaps most clearly expressed by the bold proposal of Morowitz [24,25] which plainly states that 'life started with the *reductive* citric acid cycle', implying that this type of cycle originally was capable of operating without the assistance of enzymes (Figure 13). Irrespective of the serious doubts that may be raised against the validity of this latter assumption, the merit of the proposal lies in its exemplification of how, in principle, such a metabolic cycle could act as the heart of a replicating chemical *system*. The cycle (running in the reductive direction which is constitutionally opposite to that of the contemporary citric acid cycle) would be autocatalytic, since each run through both branches of the cycle would convert input materials (CO_2 and reductants) not in one, but in two

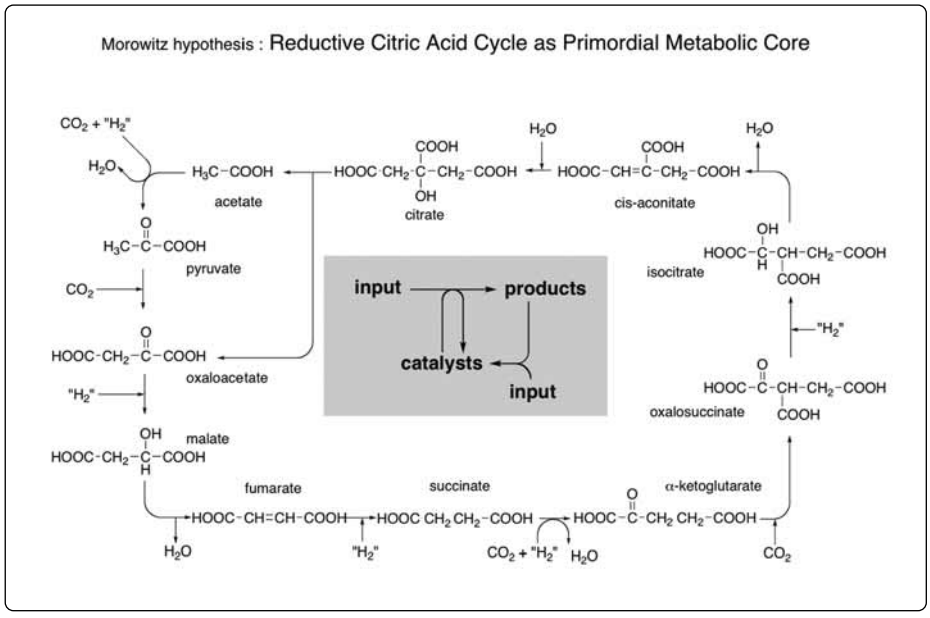
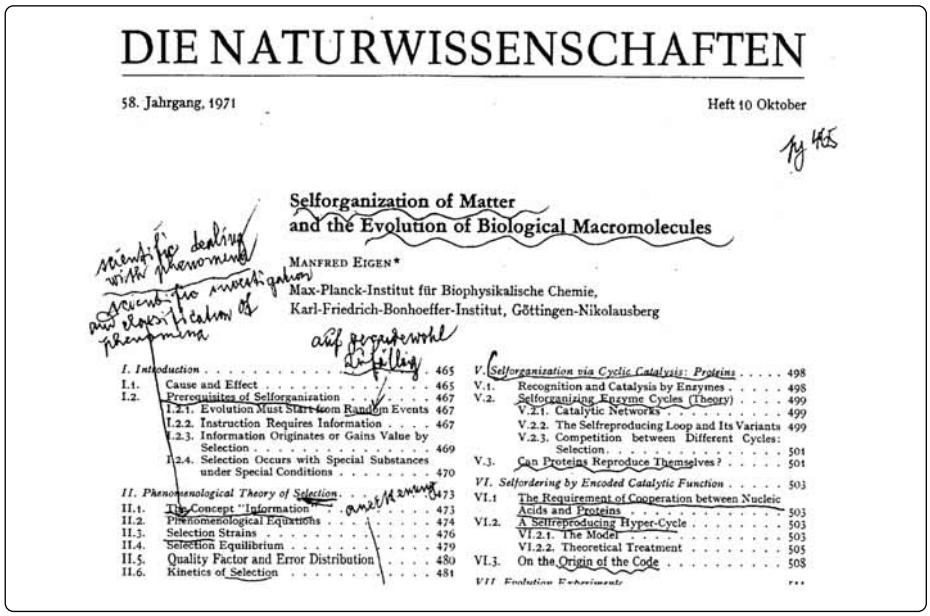


Figure 13. *Harold Morowitz*'s proposal that (a non-enzymic version of) the reductive citric acid cycle has been the seed for life's origin [24,25]. The serious doubts organic chemists may have concerning the presumed operation of such a cycle operate without enzymes notwithstanding, the reductive citric acid cycle is a good example to exemplify essentials of an autocatalytic metabolic cycle: exergonic input reactions are to drive a reaction cycle in which two equivalents of each cycle-constituents are formed in each run and in which each cycle constituent is a catalyst both for its own formation as well as for the formation of all the other constituents of the cycle.

equivalents of any given cycle constituent, and each of these constituents – by virtue of their very affiliation to the cycle – would act as catalyst for the formation of itself and of all other constituents of the cycle. Running through the cycle would amount to the self-replication of a family of catalysts. The appeal of the reductive citric acid cycle as the seed of life's origin derives from the role that enzymic versions of the cycle play in some anaerobic microorganism (CO_2 assimilation) and on the fact that the constituents of an oxidative version of the cycle proceeding in contemporary organisms plays an absolutely central role in metabolism.

In the geneticists' view, genetic function is to be assigned unequivocal supremacy over metabolic function when it comes to define the requirements for an organized chemical system to be capable of undergoing Darwinian evolution. Molecular evolvability has as its prerequisite the functioning of an oligomer system that is capable of storing, replicating, and stochastically varying structural information, whereby at least part of it (the 'genotype') must be connectable to specific catalytic functions (the 'phenotype'). The viewpoint received its theoretical inauguration in Manfred Eigen's classic publication entitled 'Self-Organization of Matter and the Evolution of Biological Macromolecules' in 1971 [17] in which for the first time the concept of evolutionary processes on the molecular level was propounded and the kinetic principles that will dominate such processes delineated in conceptual and mathematical terms. Shortly afterwards, a paper entitled 'Self-Organization of Molecular Systems and Evolution of the Genetic Apparatus' appeared, in which its author, Hans Kuhn [18], propounded and exemplified the pragmatic paradigm that the conundrum of life's origin should be approached as a physico-chemical engineering problem. Both papers, pioneering in their time and focusing on concepts, had to circumvent the specific chemical questions that from today's point of view are the central ones, namely, the questions concerning the nature of the *chemistry* of life's origin.

In contemporary living cells a molecular machinery of extraordinary structural and functional complexity, the ribosome, fulfills the extraordinarily complex task of translating – mediated by the genetic code – the *genetic* information stored in the constitutional diversity of one type of biopolymer (the nucleic acids) into a constitutionally different type of biopolymer (the proteins). If a chemist undertook the attempt to think of the chemistry of a primordial molecular machinery by which a replicating oligomer system would be capable of performing a genotype-to-phenotype translation modeled after today's ribosome function, he would run



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Selforganization of Matter and the Evolution of Biological Macromolecules

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Figure 14. The first page of *Manfred Eigen's* classic paper [17] containing handwritten personal comments made by the organic chemist *Leopold Ruzicka* (1987-1976).

into difficulties that are immense to the extent of being hopeless. This is why the notion of the '*RNA world*' [26] (Figure 15), a 'world' supposed to have preceded our 'DNA-RNA-Protein' world and one in which RNA fulfilled the functions of both the genotype and the phenotype, appears conceptually so attractive. In essence, it reduces the coding problem from (complex) chemistry to ('simple') physics in the sense of replacing an *intermolecular*ly operating chemical coding process by an i*ntramolecular* physical relationship between an oligomer molecule's *constitution* and its *conformation*. Any specific constitution (base sequence) of an RNA molecule induces the molecule to adopt a specific shape (conformation). We may say, the RNA molecule's constitution '*codes*' for that shape. In a system that could screen a population of RNA shapes (and implicitly RNA sequences) for catalytic capabilities, any RNA sequence turning out to be capable of a catalytic function that exerts a positive feedback on RNA synthesis would amount to the acquisition of a catalyst that will boost the system's survival. The concept of the RNA world implicates the capabili-

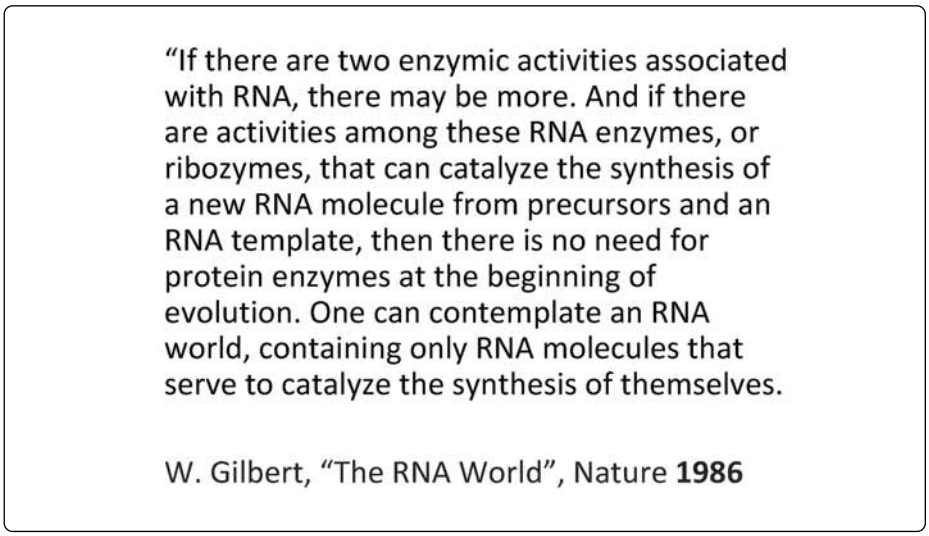

Figure 15. *Walter Gilbert's* pronouncement of the notion of 'RNA World' [26] in the wake of *Tom Ceck's* and *Sidney Altman's* discovery [29] of catalytic RNA's ('ribozymes').

ty of RNA sequences to replicate, mutate, select for RNA catalysts and, therefore, to undergo Darwinian evolution.

The important idea that RNA could originally have fulfilled both a genetic and a phenetic function had been adumbrated by F. Crick [27], L.E. Orgel [16], and C. Woese [28] as early as 1968. It became a realistic concept in 1986 [26] in the wake of the discovery of ribozymes [29]. Since then, massive support for the RNA-world concept has come from structural biology, as well as from research operating with the technique of *in vitro* evolution of RNA sequences. Comprehensive X-ray structure analyses in various laboratories revealed the structure of the (microbial) ribosome to document the surprising as well as highly significant fact that the ribosome is in essence a ribozyme, since the molecules within the ribosome that are most intimately engaged in catalyzing protein synthesis are RNA molecules and not proteins. By *in vitro* evolution (Figure 16) a host of new ribozymes have been uncovered, RNA molecules that are capable of catalyzing a large diversity of chemical reactions [30], the most dramatic of them being a specific ribozyme's own replication (see below).

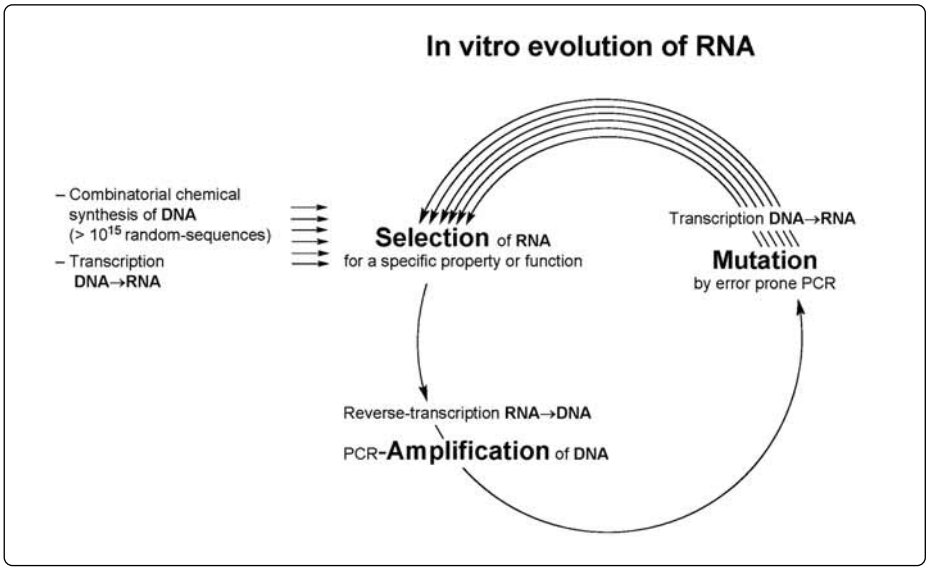

Figure 16. General principle of the experimental search for RNA sequences that fulfill a specific function (e.g. complexing with a specific biomolecules or displaying catalytic activity for a specific chemical reaction) by *in vitro* evolution [30].

The advent of the RNA-world concept had a marked impact on the thinking of researchers in the origin of life field and, at the same time, reinvigorated projects of exploring the potential of RNA to be generated under potentially prebiotic conditions. Significant progress in this direction has been made, especially on the formation of the sugar unit and certain of its nucleosides, on template-assisted oligomerization of suitably activated monomers in solution and on mineral surfaces [12]. However, no generational pathway to RNA that could be said to be potentially prebiotic has been demonstrated thus far. Much attention has been and is being devoted to providing experimental 'proofs of principle' for the feasibility of molecular replications with chemical systems under conditions not subjected to any sort of prebiotic constraints; such demonstrations have been achieved with both oligonucleotides [31] and oligopeptides [32]. Very recently, Gerald Joyce at the Scripps Institute succeeded in creating by *in vitro* RNA evolution ribozymes which, by template controlled cross-catalytic ligation of two RNA components, are capable of exponentially reproducing them-

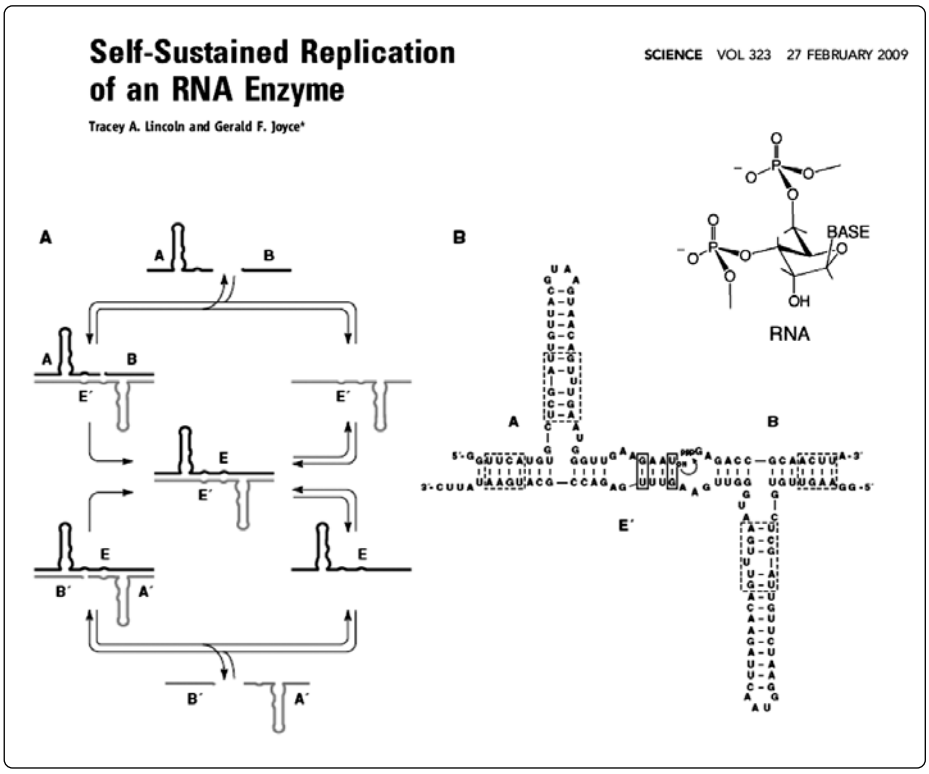

Figure 17. *Gerald Joyce*'s most recent RNA self-replication experiment [33], thus far the most advanced 'proof of principle' in support of the RNA world concept. – Scheme (A) on the left part of the Figure: A, B, E and A', B', E' denote RNA sequences. The two ribozymes (E and E') catalyze each others' synthesis from four oligonucleotide substrates (A, B, A' and B'). E' catalyzes the ligation of substrates A and B to form ribozyme E which, in turn, catalyzes ligation of corresponding substrates A' and B' to form ribozyme E'. Importantly, the duplex between the two ribozymes E and E' (center of the scheme on the left) dissociates into the single strands under the reaction conditions such that the process can repeat 'indefinitely' as long as the four substrates are provided. – Part B of the Figure: RNA sequence formula of the (transiently formed) complex between ribozyme E' and the (complementary) substrates A and B . The arrow indicates the position at which the ligation occurs by elimination of pyrophosphate (excerpt from Fig. 1 in [33]). – The chemical formula on the upper right is to remind the reader of the chemical structure of the nucleotide unit of RNA (B = Nucleobase, A or G or U or C). Note that the replication process does not require any protein enzymes and that (in principle) the RNA sequences used in the experiment can be synthesized by chemical methods.

selves without any assistance by a protein [33] (Figure 17). While this and all the earlier replication experiments are of considerable theoretical interest, they also make clear how far we still are from corresponding experiments under conditions that could be said to be compatible with the constraints of prebiotic chemistry. Figure 18 summarizes the research field's rather sobering state of the art.

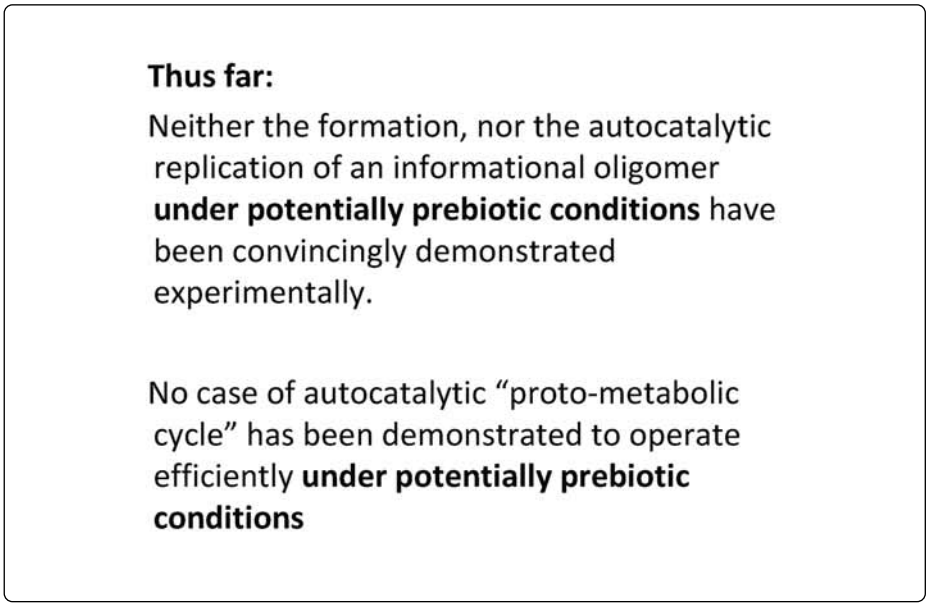

Figure 18.

The intrinsic limits any attempt of retrodicting chemical events of the past is facing will induce chemists to launch research on self-organizing chemical systems in complete independence from the environmental and geochemical constraints that the search for the chemistry of life's origin is subjected to. The quest is to think of, synthesize and study adaptive and self-sustaining chemical systems and, in the (perhaps very) long run, to create what will amount to elementary forms of *artificial chemical life*. Among those who are challenged are primarily physical-organic chemists who are prone to engage themselves in what today is recognized as the emerging field of 'systems chemistry' [34]. Its task is to deal with the wealth of prob-

lems dynamic structures of autocatalytic chemical systems are going to pose and to explore the new horizons they will open. Research toward the creation of artificial chemical life will be an important, if not the most important empirical source of knowledge for our eventual comprehension of life's origin.

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