REVIEW

What Is Life?
A Brief Historical Overview

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Dedicated to the memories of *Leslie E. Orgel* and *Stanley L. Miller*

1. *'I Sing the Body Electric'.* – After many years of experimentation on the effects of electricity on frog legs, in 1791 *Luigi Galvani* published his *Commentary on the Effects of Electricity on Muscular Motion*, summarizing the observations that had led him to believe in the existence of 'animal electricity' that originated in the brain, and traveled through nerves and muscles. A child of the Enlightenment, *Galvani* was no mystic, and the fascination that his observations awoke in both his colleagues and the lay public (which are echoed in *Mary Wollstonecraft Shelley*'s masterpiece *Frankenstein*) must be understood as part of the process of secularization that life sciences underwent through this period: *Galvani* was, in fact, attempting to explain the nature of life itself on the basis of a purely physical phenomenon. As shown by the 19th century efforts to describe the basic properties of life on the basis of magnetism, surface tension, radioactivity, and other physical phenomena [1], *Galvani* and others had initiated a scientific trend that has continued for over two centuries.

In a way, *Erwin Schroedinger*'s famous book *What is life?* [2] can be seen as part of this trend. *Schroedinger*'s text should be read not as the starting point of the appeal that biological phenomena had over many physicists, but rather as the culmination of a long tradition that attempted to explain the nature of life in physical terms. What is generally not realized is that *Schroedinger* did not include in his book a single reference to biology. This is quite surprising, especially since many of his contemporaries were already having important insights when addressing basic properties of life such as heredity. A few years before *What is life?* was published, for instance, *John B. S. Haldane* wrote that '…*two possibilities are now open. The gene is a catalyst making a particular antigen, or the antigen is simply the gene or part of it let loose from its connection with the chromosome. The gene has two properties. It intervenes in metabolism, sometimes at least by making a definitive substance. And it reproduces itself. The gene, considered as a molecule, must be spread out in a layer one building block deep. Otherwise it could not be copied. The most likely method of copying is by a process analogous to crystallization, a second similar layer of building block being laid down on the first. But we could conceive of a process analogous to the copying of a gramophone record by the intermediation of a 'negative' perhaps related to the original as an antibody to an antigen…*' [3].

2. From Primordial Enzymes to Ancestral Nucleic Acids? – The same year that *Schroedinger*'s book appeared, the seminal paper by *Avery*, *McLeod*, and *McCarty* [4] on the role of nucleic acids in hereditary phenomena was also published. Its publication marks the starting point of the extraordinary developments of molecular biology that have firmly established the central role that nucleic acids have in all cells. Perhaps not surprisingly, its publication would eventually lead to the reinforcement of previous attempts to define life solely in genetic terms. In a series of papers published during the First World War, the American physicist *Leonard Troland* [5–7] had argued the origin of life was due to the random formation of a self-replicating enzyme-like molecule that had made its sudden appearance in the primitive oceans. A few years later *Hermann J. Muller*, an American geneticist who would play an important role in the understanding of *Mendelian* heredity, explicitly adapted *Troland*'s hypothesis to propose that the ancestral molecule had been, in fact, a gene [8].

The evolution of *Muller*'s ideas on the nature of life ran parallel to the molecularization of biology that would dominate research for many decades. The double-helix model of DNA and the success in prebiotic syntheses led him to update his ideas by arguing that what had emerged in the primitive oceans had been, in fact, a primordial DNA molecule: '…*it is to be expected that at last, just before the appearance of life, the very ocean had become, in* Haldane's [9][10] *vivid phraseology, a gigantic bowl of soup*', wrote *Muller*, and added '*drop into this a nucleotide chain and in should eventually breed*!' [11]. A few years later he would add '…*life as we know it, if stripped of all its superstructures, lies in the three faculties possessed by the gene material. These may be defined as, firstly, the self-specification, after its own pattern, of new material produced by it or under its guidance; secondly, of performing this operation even when it itself has undergone a great succession of permanent pattern changes which, taken in their totality, can be of a practically unlimited diversity; thirdly, of, through these changes, significantly and (for different cases) diversely affecting other materials and, therewith, its own success in genetic survival*' [12]. In other words, the essence of life lies in the combination of autocatalysis, heterocatalysis, and mutability, *i.e.*, evolvability. '*The gene material alone, of all natural materials*', wrote *Muller* [12] '*possesses these faculties, and it is therefore legitimate to call it living material, the present-day representative of the first life*'.

3. Genes *vs.* Protoplasm. – *Muller*'s hypothesis was brilliantly reductionist, and was soon contested by *Alexandr I. Oparin* and others in a now largely forgotten debate. Their controversy became an entangled debate in which science, philosophy, and politics mixed in an excruciating discussion that was shaped in part by the Cold War atmosphere [13–15]. In sharp contrast with *Muller*'s ideas, *Oparin* [16] argued that the essence of life was metabolic flow. For him, life is '*a special form of the motion of matter*', always in flow, which included enzymatically based assimilation, growth, and reproduction, but not nucleic acids, whose genetic role was not even suspected during the 1930's. Biological inheritance was assumed by *Oparin* to be the outcome of growth and division of the coacervate drops he had suggested as models of precellular systems, a view that led *Muller* [12] to write that '*the Russian* Oparin *has since the early 1930's espoused this view and has followed the official Communist Party line by giving the specific genetic material a back seat*'.

Oparin and *Muller* came from different scientific backgrounds and almost opposite intellectual traditions. Their common interest in the origin of life did nothing to assuage their opposing views. *Oparin* was a convinced evolutionist, and, like many of his contemporaries, his original genetics were pre-*Mendelian*. *Oparin*'s *Darwinism* had been nurtured by *Kliment A. Timiriazev*, who had famously declared in 1912, many years before the *Lysenko* affair, that 'the Mendelians *and mutationists*' were the enemies to be defeated in the war against '*anti*-Darwinism' [17]. For *Muller*, life could be so well-defined that the exact point at which it started could be established with the sudden appearance of the first DNA molecule. *Oparin*, on the other hand, refused to admit that life could arise all at once by a spontaneous generation, and argued that it was the outcome of a slow, stepwise evolutionary developmental process. His ideas were first published in 1924, in a small booklet which can be read as the work of a young, bold, and talented researcher plenty of enthusiasm and free of intellectual prejudices. It is this first book where *Oparin* first argued that what is truly unique about life is that all properties that characterize it are found in the same entity [18]. In retrospect, this first book can be considered the harbinger of his major work, a 1936 Russian volume also called *Origin of Life*, whose English translation became available two years later [16].

Perhaps the most important scientific achievement of *Oparin* was the methodological breakthrough that transformed the study of the origin of life from more a purely speculative problem into a workable multidisciplinary research program, brilliantly summarized in his 1938 book. As *Farley* [14] wrote, this volume '*is probably the most significant book ever published on the origin of life*'. Many workers in the field are aware that many of *Oparin*'s original ideas have been superseded, leading to changes that have improved the postulates and assumptions underlying his initial hypothesis. Over the years, however, it has become clear that the open character of his theory of chemical evolution has allowed the incorporation of new discoveries and the development of more accurate descriptions of possible primitive scenarios without destroying its overall structure and premises [19].

4. Can Life Be Defined? – The spectacular developments in our understanding of the molecular basis that underline biological phenomena have not led to a generally agreed definition of life, and not for want of trying (see, *e.g.*, [20][21]). As argued by *Cleland* and *Chyba* [22], attempts to find a definition of life may be a useless endeavor bound to fail. This pessimism is not altogether surprising: as *Nietzsche* once wrote, there are concepts that can be defined, whereas others only have a history. Precise definitions are achievable in mathematics (*i.e.*, an imaginary number) but, as argued by *Immanuel Kant*, empirical concepts such as 'life' can only be made explicit (*cf.* [23]), in ways that are strongly dependent on historical circumstances.

Attempts to address the definition of living systems have often led to nothing more than phenomenological characterizations of life, which are often reduced to a mere list of observed (or inferred) properties. These inventories are not only unsatisfactory from an epistemological viewpoint, but may also become easily outdated and may fail to provide criteria by which life (and its traces) can be defined or recognized [24]. The lack of a precise definition of life and the parameters that characterize it often poses a serious problem, as shown, for instance, by the heated debates on the

ultimate nature of the microscopic structures in the Martian meteorite *Allan Hills 84001*.

5. Autopoiesis and Living Systems. – Since the 19th century, metabolism has been recognized as a central trait of life, a conclusion that has led to consider viruses and other subcellular biological entities as nonliving. The observation that life's continuous production of itself is based on networks of anabolic/catabolic reactions and energy flow led *Maturana* and *Varela* [25] to define life as an autopoietic system, *i.e.*, as an entity defined by an internal process of self-maintenance and self-generation. As shown by *Bernal*'s statement [26] that life is '*…the embodiment within a certain volume of self-maintaining chemical processes*', the idea of autopoiesis is not without historical precedents.

However, it is during the past decade that the idea of autopoiesis has found supporters from a wide range of scientific fields. Although it has been argued that autopoiesis refers to and is limited to minimal life forms [27], cells and organisms made of cells also fit the definition of autopoietic entities. Multicellular organisms consist of units that are living systems in themselves, and will remain so even if the entire system is destroyed [28], as demonstrated, for instance, by the extraordinary success of organ transplants. Both single-cell and multicellular organisms metabolize continuously, and, as shown by manifold biogeochemical cycles, in doing so they have changed the composition of their surroundings at a planetary scale [29].

There are a number of physical and chemical analogues that have been considered autopoietic, and that mimic some of the basic properties of life. One of the most enticing examples is that of the self-replicating micelles and liposomes described by *Pier Luigi Luisi* and his associates. In one surprising example, synthetic vesicles formed by caprylic acid containing lithium hydroxide and stabilized by an octanoic acid derivative have been shown to catalyze the hydrolysis of ethyl caprylate. The resulting caprylic acid is incorporated into the micelle walls, leading to their growth and, eventually, to their fragmentation, during several 'generations' of vesicles [30].

But neither replication nor autopoiesis suffice by themselves to define life. However surprising, replicative micelles and liposomes do not exhibit genealogy or phylogeny. Nor do prions, whose multiplication can be explained as self-perpetuating structural states involving only the transmission of phenotypes but not of genetic information. These systems replicate without transmission of information, *i.e.*, they lack heredity [31]. This is in sharp contrast to living beings: organisms may be recognized as the ultimate example of autopoietic systems [29], but the properties that underlie their self-sustaining abilities are the outcome of historical processes, making it somewhat difficult to accept a definition of life that lacks a *Darwinian* framework. Regardless of their complexity, all living beings have been shaped by a lengthy (but not necessarily slow) evolutionary history. Since life appears to be neither the outcome of a miracle nor of a rare chance event, proper understanding of the minimal properties required for a system to be considered alive requires the recognition of the evolutionary processes that led to it. In other words, the appearance of life was marked by the transition from purely chemical reactions to autonomous, self-replicating molecular entities capable of evolving by natural selection. Is it possible to establish a point in time when the difference between a chemical system and the truly primordial, first organism, took place?

6. The Primitive Soup and the Appearance of Life. – Although *Darwin* was reluctant to address in public the question of the origin of life, the possibility that living organisms were the evolutionary outcome of the gradual transformation of lifeless matter became widespread soon after the 1859 publication of *The Origin of Species*. Authors like *Pflügger*, *Hensen*, *Allen*, *Löw*, and others attempted to explain the origin of life by introducing principles of historical explanation. It is, therefore, not surprising that terms like 'primordial protoplasm' are found in 19th-century sources; from 1840 to the turn of the 20th century, several ideas on the nature of protoplasm coexisted, but with different and sometimes even opposing meanings [32][33]. Study of life's defining properties was understood by many as the physico-chemical characterization of protoplasm, and thus became part of colloid chemistry [34]. It is also well-known that, in some cases, attempts to understand the appearance of life led to a wide variety of laboratory models of protoplasm (such as those proposed by *Jerome Alexander*, *Stéphane Leduc*, and *Alfonso L. Herrera*, for instance), some of which rank, from a modern perspective, well-within the absurd [35].

Trained both as a plant biochemist and as an evolutionary biologist, for *Oparin* it was impossible to reconcile his *Darwinian* credence in a gradual, slow evolution from the simple to the complex, with the commonly held suggestion that life had emerged already endowed with an autotrophic metabolism that included photosynthetic pigments, enzymes, and the ability to synthesize organic compounds from CO_2. Like many others, *Oparin* [36] accepted the idea of primordial protoplasm but proposed that life had been preceded by a lengthy period of abiotic syntheses and accumulation of organic compounds that had led to the accumulation of the so-called primitive broth. A similar scheme was suggested a few years later by *Haldane* [9], albeit with important differences, which included the assumption that viruses represented an intermediate step in the transition from the prebiotic soup to the first heterotrophic cells. Based on the simplicity and ubiquity of fermentative reactions, and on a detailed analysis of chemical synthesis and astronomical observations, *Oparin* attempted a theoretical reconstruction of the conditions of the primitive Earth and the evolution of organic molecules into precellular systems, from which anaerobic cells that nourished themselves from the soup had evolved.

Oparin's scheme required the abiotic synthesis and accumulation of organic compounds as a prerequisite to the emergence of life. The birth and development of organic chemistry during the 19th century provided evidence that such processes were feasible. In 1827, *Jöns Jakob Berzelius*, probably the most influential chemist of his day had written that '*art cannot combine the elements of inorganic matter in the manner of living nature*'. However, one year later his friend and former student *Friedrich Wöhler* demonstrated that urea could be formed in high yield by heating ammonium cyanate '*without the need of an animal kidney*', under conditions which would qualify today as prebiotic. It is possible that the laboratory formation of urea may have been preceded by other syntheses relevant to our understanding of the origin of life. In 1807, *Joseph Louis Proust*, a French chemist who taught in Spain thanks to recommendations provided by *Lavoisier* himself, reported that under basic conditions hydrogen cyanide (HCN) produced a complex polymer, together with other uncharacterized compounds which may have included adenine [37].

Wöhler's work led to a new era in chemical research: in 1850, the German chemist *Adolph Strecker* achieved the laboratory synthesis of alanine from a mixture of acetaldehyde, ammonia, and hydrogen cyanide. This was followed by the experiments of *Alexandr M. Butlerov* showing that the treatment of formaldehyde with strong alkaline catalysts, such as sodium hydroxide, leads to the synthesis of sugars, *i.e.*, the so-called formose reaction. The laboratory synthesis of biochemical monomers soon included more complex experimental settings. Towards the end of the 19th century, organic synthesis was a solid, well-established field that had achieved the abiotic formation of many compounds, including fatty acids and sugars, using electric discharges with various gas mixtures. This work continued in the 20th century with the work of *Walther Löb*, *Oskar Baudish*, and others on the synthesis of amino acids by exposing wet formamide to a silent electrical discharge and to UV light. However, since it was generally assumed that the first life forms had been autotrophic, plant-like microorganisms, the abiotic synthesis of organic compounds did not appear to be a necessary prerequisite for the emergence of life. These organic syntheses were not conceived as laboratory simulations of the primitive Earth, but attempts to understand the autotrophic mechanisms of nitrogen assimilation and CO_2 fixation in green plants [38].

The starting point of prebiotic chemistry is the 1953 *Miller–Urey* experiment, and the laboratory simulations that followed it soon led to the demonstration that many other monomers of biochemical significance could be readily synthesized under putative primitive conditions. Indeed, the easiness of formation, under reducing atmospheres, of amino acids, purines, and pyrimidines strongly suggest that these molecules were components of the prebiotic broth. Company would have been kept by many other compounds, such as urea and carboxylic acids, sugars formed by the nonenzymatic condensation of formaldehyde, a wide variety of aliphatic and aromatic hydrocarbons, alcohols, and branched and straight fatty acids, including some which are known to form bilayered membranes [39].

The collisions of cometary nuclei against the primitive Earth combined with the contribution from other extraterrestrial bodies such as meteorites and interplanetary dust may have spiced the primitive broth with extraterrestrial organic compounds [40]. Regardless of their ultimate origin, simple organic molecules dissolved in the primitive oceans or other bodies of water would need to be concentrated and polymerized by simple physicochemical mechanisms. How these simple organic constituents were assembled into polymers and then into the first living entities is currently the most challenging area of research in the origin of life.

7. Pyrite-Based Life? – Some fifteen years ago *Günter Wächtershäuser* [41] suggested that life began with the appearance of an autocatalytic two-dimensional metabolic system based on autotrophic fixation of CO_2 and the formation of the highly insoluble mineral pyrite (FeS_2). There are a number of experiments that have demonstrated *Wächtershäuser*'s insightful prediction that ferrous sulfide in the presence of hydrogen sulfide (H_2S) is an efficient reducing agent: pyrite formation can produce molecular hydrogen, promote the formation of ammonia from nitrogen, and can reduce a few organic molecules under mild conditions. Compared with the surprising variety of biochemical compounds that are readily synthesized in one-pot

simulation experiments like that performed by *Miller* [42], the suite of molecules produced under the conditions suggested by *Wächtershäuser* is quite limited. However, the impressive demonstration that FeS/H_2S can reduce nitrogen to ammonia [43] shows that considerable attention should be given to the reducing power of pyrite formation under possible prebiotic conditions.

The lack of precise definitions of synonymous terms like 'primitive soup', 'primordial broth' or '*Darwin*'s warm small pond' has led to major misunderstandings, including the simplistic image of a worldwide tarry ocean rich in self-replicating molecules accompanied by all sorts of biochemical building blocks ready to be incorporated into prokaryotic-like protobionts. The term '*Darwin*'s small pond', which has long be used for convenience (and is thus equivalent, for instance, to *Newton*'s apple, *Maxwell*'s demon, or *Schroedinger*'s cat), refers not necessarily to the entire ocean, but those parts of the hydrosphere where the abiotic synthesis, accumulation and interaction or organic compounds, may have taken place, including oceanic sediments, fresh water lakes, eutectic environments, small ponds undergoing wet-and-dry cycles and, of course, pyrite-rich environments.

However, the empirical support for some of *Wächtershäuser*'s central tenets is meager. Life does not consist solely of metabolic cycles, and there is no evidence from the experiments performed so far that proves that enzymes and nucleic acids are the evolutionary outcome of multistep autocatalytic metabolic cycles surface-bounded to pyrite or some other mineral. In judging the different proposals on the origin of life (some of which clearly strain our imagination), it is useful to recall what *John D. Bernal* wrote in his 1951 *The Physical Basis of Life:*

'*In a letter to* Sir J. Hooker, [Darwin] *said: 'It is mere rubbish thinking at present of the origin of life; one might as well think of the origin of matter…', this does not mean that we should accept wild hypothesis of the origin of life or of matter, which simply conceal ignorance, but rather that we should attempt almost from the outset to produce careful and logical sequences in which we can hope to demonstrate that certain stages must have preceded certain others, and from these partial sequences gradually built up one coherent history. There are bound to be gaps where this cannot be done, but until the process is attempted these gaps cannot be located, nor can the attempt be made to fill them up…*' [44].

8. The Search for an RNA World. – The *Miller* paper [42] was published only a few weeks after the *Watson* and *Crick*'s [45] classical article on the DNA helix model structure appeared. With few exceptions, modern attempts to understand the origin of life have been shaped by the unraveling of the details of DNA replication and protein biosynthesis. The weak connection between origin-of-life studies and molecular biology started with the prebiotic synthesis of adenine and other purines [46], and was enhanced with the experiments by *Leslie Orgel* on enzyme-free template-dependent polymerization, but reached its highlight with the discovery and development of the catalytic activity of RNA molecules, reinforcing the idea of the 'RNA world' – a hypothetical stage before the development of proteins and DNA genomes during which alternative life forms based on ribozymes existed [47–49].

The origin of genetic polymer replication remains a major, unsolved problem. However, the study on the nonenzymatic template-directed synthesis of RNA started

by *Leslie Orgel* led to the synthesis of oligo(G)s on a poly(C) template [50]. The development of an aqueous phase system based on the 2-methylimidazole-activated guanosine resulted in the formation of oligo(G)s by template-directed synthesis containing predominantly 3′,5′-linked phosphodiester bonds [51]. The actual chemical nature of the ancestral genetic polymer backbones is, of course, unknown, but the *Orgel*'s experiments are a striking demonstration that template-directed polymer-izations and the transfer of genetic information could have taken place in the absence of enzymes.

The appearance of first self-sustaining entities capable of replication, catalysis, and multiplication with variation would have been essential for the origin of both life and evolution. What were the fundamental characteristics of these first molecular living entities that distinguished them from nonliving chemistry? It is very unlikely that the RNA world would have arisen directly from the primitive oceans. *Butlerow*'s experiments had demonstrated that the autocatalytic condensation of formaldehyde under basic conditions and in the presence of small amounts of glycolaldehyde produces a wide variety of sugars that include ribose, which is part of the RNA backbone. However, ribose is a very labile molecule that decomposes rapidly, especially at high temperatures. Recent experiments have demonstrated that it can be stabilized by boron [52][53] and by cyanamide [54], and that, under *in vitro* conditions, is more likely to cross bilayer membranes than other carbohydrates [55]. These data provide important insights into why ribose may have been eventually selected as a component of RNA. However, the high number of possible random combinations of derivatives of nucleobases, sugars, and phosphate that may have been present in the prebiotic soup make it unlikely that an RNA molecule capable of catalyzing its own self-replication arose spontaneously.

These difficulties have led to the suggestion that the RNA world was not a direct product of abiotic processes, but may have been the evolutionary outcome of some predecessor primordial living systems of what are now referred to as pre-RNA worlds. The chemical nature of the first genetic polymers and the catalytic agents that may have formed the hypothetical pre-RNA worlds that may have bridged the gap between the prebiotic broth and the RNA world are completely unknown and can only be surmised. Modified nucleic acid backbones with a different version of ribose or endowed with simpler sugars have been synthesized by *Albert Eschenmoser* and his associates. Like other numerous double-stranded polymeric structures with backbones quite different from those of nucleic acids, these were held together by *Watson–Crick* base pairing [56]. Other nitrogen base-bearing polymers can be formed that lack ribose and phosphate altogether, like the simple alternating peptides based on simple D- and L-amino acids that form stable antiparallel *Watson–Crick* hydrogen-bonded double helices [57]. The search for models of pre-RNA worlds does not imply that genetic polymers could only evolve from simpler genetic polymers in a never-ending succession of genetic takeovers, but should be seen as a research program to study, under plausible prebiotic conditions, very simple monomers and genetic polymers that could serve as laboratory models of the possible evolutionary precursors of RNA.

9. Life and the Single, Lonely Molecule. – The lack of an all-embracing, generally agreed definition of life sometimes gives the impression that what is meant by its origin

is defined in somewhat imprecise terms, and that several entirely different questions are often confused. For instance, until a few years ago the origin of the genetic code and of protein synthesis was considered synonymous with the appearance of life itself. This is no longer a dominant point of view: four of the central reactions involved in protein biosynthesis are catalyzed by ribozymes, and their complementary nature suggest that they first appeared in an RNA world, *i.e.*, that ribosome-catalyzed, nucleic acid-coded protein synthesis is the outcome of *Darwinian* selection of RNA-based biological systems, and not of mere physico-chemical interactions that took place in the prebiotic environment [58].

The catalytic versatility of RNA molecules clearly merits a critical reassessment of *Muller*'s ideas [8][12], but there are many different definitions of what the RNA world was. The discovery of ribozymes does not imply that wriggling autocatalytic nucleic acid molecules ready to be used as primordial genes were floating in the waters of the primitive oceans, or that the RNA world sprung completely assembled from simple precursors present in the prebiotic soup. In other words, the genetic-first approach to life's emergence does not necessarily imply that the first replicating genetic polymers arose spontaneously from an unorganized prebiotic organic broth due to an extremely improbable accident, or that the precellular evolution was a continuous, unbroken chain of progressive transformations steadily proceeding to the first living beings.

The assumption that a single type of molecule once served both as the depositary of information storage and as biological catalyst is not necessarily married to a reductionist approach that assumes that life can be assigned to such compound. Many prebiotic *culs-de-sac* and false starts probably took place, with natural selection acting over populations of primordial systems based on genetic polymers simpler than RNA, in which company must have been kept by a large number of additional organic components such as amino acids, lipids, and sugars of prebiotic origin, as well as a complex assemblies of clays, metallic ions, *etc*. What we lack is a laboratory model or a detailed theoretical scheme indicating how to go from the primitive soup to the RNA world.

10. Complexity and the Appearance of Life. – According to some, life can be understood as a self-maintaining emergent property of complex systems that may have started with the appearance of self-assembled autocatalytic metabolic networks initially lacking genetic polymers [59]. Life should then be seen as an emergent interactive system endowed with dynamic properties that exist in a state close to chaotic behavior, *i.e.*, as a self-sustaining cycle of reactions that resulted from an spontaneous phase transition of a complex chemical system with a critical mass of diverse molecules, each of which was endowed with the ability to catalyze a reaction.

This is somewhat similar to the idea of autopoiesis. It is true that many examples of self-organization and complex systems can be found in biology, including the self-assembly of biological membranes and of viral capsids and, at a macroscopic level, the organized distribution of birds in swarms and fishes in schools. Self-assembly and complexity are not unique to biology, and may be found in a wide variety of systems, including cellular automata, the complex flow patterns of many different fluids such as tornadoes, cyclic chemical phenomena (including reaction such as those described by *Belousov–Zhabotinsky* and *Briggs–Rauscher*), as well as in the auto-organization of lipidic molecules in bilayers, micelles, and liposomes.

A number of observations, such as the assembly of lipids into the bilayered membranes do not require genes (but their synthesis does, as shown by the number of sequences encoding the different steps leading to the biosynthesis of lipids) supports the idea that self-organizing phenomena are important for biology. It is also true that, under given conditions, the self-organization of lipidic molecules into liposomes, for instance, can lead to the spontaneous formation of microenvironments which may have had significant roles in the emergence of life. But they are not alive, even if they replicate.

According to *Kauffman* [59], life's crystallization takes place when the phase transition of closed networks of self-sustaining, replicative sets of polymers gathered at random reach a critical complexity level and replicate as a whole leading to cycles of progressively larger molecules. Accordingly, the emergence of the origin of life should be understood as the origin of replicative complex metabolic network sustained by raw materials and energy provided by the environment. This is an awfully big assumption, and as of today there is no evidence to support it. Prebiotic organic compounds very likely underwent many complex transformations, but there is no evidence that metabolic cycles could spontaneously self-organize, much less replicate, mutate, and evolve.

Theories that advocate the emergence of complex, self-organized biochemical cycles in the absence of genetic material are hindered not only by the lack of empirical evidence, but also by a number of unrealistic assumptions about the properties of minerals and other catalysts required to spontaneously organize such sets of chemical reactions. As of today, the only known prebiotic example is the formose reaction [60]. However, complex systems of chemical reactions such as the formose reaction are not adapted to ensure their own survival and reproduction – they just exist. Life cannot be reduced to one single molecule such as DNA or a population of replicating ribozymes, but current biology indicates that it could have not evolved in the absence of a genetic replicating mechanism insuring the stability and diversification of its basic components.

The many examples of self-organizing physical systems that lead to highly ordered structures shows that, in addition to natural selection, there are other mechanisms of ordered complexity that operate. However, when complexity theory and the *Darwinian* viewpoint are placed side by side, something clearly does not add up. There are some common features among a variety of different self-organized systems, and it has been claimed by a number of theoreticians that they follow general principles that are, in fact, equivalent to universal laws of nature. Perhaps this is true. The problem is that the evidence for such all-encompassing principles, if they exist at all, has so far remained undiscovered [61]. This has not stopped a number of overeager researchers to use complexity theory embellished with mathematical formulae to attempt to explain life as a continuously renewing interactive system that emerged as self-organizing metabolic cycles that did not require genetic polymers.

If self-sustaining reaction chains did arise on the early Earth, they could have played an important role in enriching the prebiotic soup in components not readily synthesized by other abiotic reactions or delivered from space. There are worthy attempts to reconcile metabolism-first with genetic-first models of the origin of life [62]. What is lacking is the confirmation that metabolic (or protometabolic) routes can replicate and evolve. Evidence for the spontaneous origin of catalytic system

and of metabolic network replication would indeed be exciting – if it could be established.

It is easy to recognize that the current attempts to explain the nature of life on the basis of complexity theory and self-assembly phenomena fall into a long and somewhat erratic intellectual tradition that has led physicists to search for all encompassing laws that can be part of grand theory encompassing many, if not all, complex systems [1]. Unfortunately, complexity models have promised much but have delivered little, and, in some cases, invocations to spontaneous generation appear to be lurking behind appeals to undefined 'emergent properties' or 'self-organizing principles' that are used as the basis for what many life scientists see as grand, sweeping generalizations with little, if any, relationship to actual biological phenomena [63].

11. Towards a *Darwinian* Definition of Life. – Following his 1946 conversations with *Einstein* in Princeton on the underlying biochemical unity of the biosphere, *John D. Bernal* wrote that '…*life involved another element, logically different from those occurring in physics at that time, by no means a mystical one, but an element of* history. *The phenomena of biology must be…contingent on events. In consequence, the unity of life is part of the history of life and, consequently, is involved in its origin*' [64].

The chronicles of Rome, the Vatican, and the Aztec empire with their long lists of unrelated Roman emperors, of Popes, and of Aztec *tlatoanis*, respectively, demonstrate that there can be historical continuity with no genetic inheritance. However, in biology, history implies genealogy and, in the long term, phylogeny. This requires an intracellular genetic apparatus able to store, express, and, upon reproduction, transmit to its progeny information capable of undergoing evolutionary change. All the available evidence indicates that the most likely candidates for this appear to be genetic polymers.

A good case can thus be made that *Darwinian* evolution is essential for understanding the nature of life itself. Accordingly, life could be defined as a self-sustaining chemical system (*i.e.*, one that turns resources into its own building blocks) that is capable of undergoing *Darwinian* evolution (*cf.* [65]). Such tentative definition, which was the outcome of a discussion group convened by NASA in the early 1990's, has been rejected by a number of authors who argue on different grounds that a single definition is impossible [22][66]. It is true that no single parameter suffices by itself to define life, but, since evolutionary change due to natural selection acting upon a system replicating with variation is indeed a unique feature of living systems, their basic nature cannot be understood without it.

The suggestion that life can be understood as a self-sustaining chemical system capable of undergoing *Darwinian* evolution is of course consistent with the well-known fact that cyanobacteria, plants, and other autotrophs are not only self-sustaining (and, by definition, autopoietic), but also very much alive. But what about the first life forms? Clearly, if, at its very beginning, life was already a self-sustaining entity capable of turning external resources into its own building blocks, then it must have been endowed with primordial metabolic routes that allowed it to use as precursors environmental raw materials (such as CO_2 and N_2, for instance). This appears unlikely to many biologists. An alternative possibility is that the first living entities were systems capable of undergoing *Darwinian* evolution (*i.e.*, endowed with genetic material

capable of replication, change, and heredity) whose self-sustaining properties depended on the availability of organic molecules already present in the primitive environment. This conclusion is, in fact, fully consistent with an updated hypothesis of the prebiotic soup and the heterotrophic origin of life [67].

12. Conclusions. – Is fire alive? *Tolstoy* has written what may be one of the most enticing analogies between life and fire. As *Anna Karenina* is dying in the train station, '*the candle by which she had been reading the book*' wrote *Tolstoy*, '*filled with trouble and deceit, sorrow and evil, flared up with a brighter light, illuminating for her everything that before had been enshrouded in darkness, flickered, grew dim and went out for ever*'. Is fire alive? Like life, fire can grow, multiply, and exchange matter and energy with its surroundings. A flame begets another flame. As shown by the way bearers of the Olympic torches transmit their flame from runner to runner, fire can have a history. But it has no heredity [68] and, therefore, no genealogy. In contrast with fire, tornadoes, replicative micelles, and other non-living systems, the history of life is recorded in its molecular constituents.

It is true that living systems are autopoietic, self-organized replicative systems. It is equally true that many properties associated with cells are observed in non-biological systems, such as catalysis, template-directed polymerization reactions, and self-assemblage. As noted by *Morange* [18], *Oparin* [36] was the first to underline this peculiar fact in his attempt to define life. '*The specific peculiarity of living organisms is only that in them there have been collected and integrated an extremely complicated combination of a large number of properties and characteristics which are present in isolation in various dead, inorganic bodies*' wrote *Oparin* [36]. '*Life is not characterized by any special properties but by a definite, specific combination of these properties*'. This implies, of course, that life cannot be defined on the basis of a single property or substance, and suggests that the appearance of living systems was the outcome of the synchronic emergence and coevolution of their basic components.

If the origin of life is seen as the evolutionary transition between the nonliving and the living, then it is meaningless to attempt to draw a strict line between these two worlds. The appearance of life on Earth should, therefore, be seen as an evolutionary continuum that seamlessly joins the prebiotic synthesis and accumulation of organic molecules in the primitive environment, with the emergence of self-sustaining, replicative chemical systems capable of undergoing *Darwinian* evolution. Instead of engaging in footling arguments about when exactly did life start, the recognition that it is the outcome of an evolutionary process can lead to the acceptance that the properties associated with living systems, such as replication, self-assemblage, or catalysis are also found in nonliving entities.

Research in the origin and nature of life is doomed to remain, at the best, as work in progress. It is difficult to find a definition of life accepted by all, but the history of biology has shown that some efforts are much more fruitful than others. It is easy to understand the appeal of autopoiesis and complexity theory when attempting to describe the basic nature of living systems. However, there is no evidence indicating how a system of large or small molecules can spontaneously arise, and evolve into non-genetic catalytic networks, and there is a major distinction between purely physico-chemical evolution and natural selection, which is one of the hallmarks of biology. In

spite of many published speculations, life cannot be understood in the absence of genetic material and *Darwinian* evolution. As *Gould* [69] once wrote, to understand the nature of life, we must recognize both the limits imposed by the laws of physics and chemistry, as well as history's contingency.

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