

Origin of Life Scenarios: Between Fantastic Luck and Marvelous Fine-Tuning

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Abstract

The unequivocal sign of creativity in science is the emergence of previously unrecognized links between facts, concepts, strategies and goals. Observations and speculations become real discoveries as they partake in a network of conceptual implications, thus becoming significant to knowledge. The anticipation of acquiring new beneficial knowledge has always motivated the work of scientists and spurred unconventional thinking, often leading to scientific discoveries that have affected our perception of reality, nature and life. The desire of new revolutionary, paradigm-breaking understanding pushes science toward topics relevant for our metaphysical or even religious perspective on reality: the boundaries of the cosmos, consciousness, the constituents of matter, the destiny of the universe and so on. In this article we offer a short description of the state of the art in the origin of life research and describe examples of creative thinking in this field. We will also discuss the far reaching implications of the direction underlying the most recent research efforts and visions. The occasion for this discussion is given by the recent finding involving the authors, of a new mechanism of molecular self-association: namely, the self-assembly of extremely short fragments of DNA or RNA into large scale ordered structures which could help explaining the prebiotic formation of polymers.

1. Introduction

The desire of new revolutionary, paradigm-breaking understanding pushes science toward topics relevant for our metaphysical or even religious perspective on reality: the boundaries of the cosmos, consciousness, the constituents of matter, the destiny of the universe and so on. This contribution focuses on one of these topics having far-reaching implications: the origin of life (OL). Humans have always been striving for knowing the mystery beyond their own existence and the essence of life in general. This tension comes from the awareness - woven into the roots of our thinking - that our very existence cannot be thoroughly understood and that its investigation may reveal the fundamental secrets of life and being. The investigation around the OL, despite being only a small part in this basic human endeavor, is fully loaded with its tension.

In this article we offer a short description of the state of the art in the OL research and describe examples of creative thinking in this field. We will also discuss the far reaching implications of the direction underlying the most recent research efforts and visions. The occasion for this discussion is given by the recent finding involving the authors, of a new mechanism of molecular self-association: namely, the self-assembly of extremely short fragments of DNA or RNA into large scale ordered structures which could help explaining the prebiotic formation of polymers. Whether or not it will turn into a convincing piece of the prebiotic events, this new mechanism is a good example of the direction that research has adopted in this field, and an occasion to better understand the interplay between creativity and expectation.

Past the season of enthusiasm for Miller's discovery of the abiotic synthesis of simple organic compounds, the growing awareness that random chemistry couldn't have assembled functional biomolecules and the feeling of the existence of an unknown mechanism have stimulated creative thinking in a wide community of chemists, biologists, physicists and geologists. This effort generated a few hypothetical scenarios for the origin of systems capable of evolving through selection. Quite interestingly, these scenarios are generally based on molecular self-assembly. Indeed, the concept that molecules can spontaneously associate in structures of various forms is currently acting as an "attractor" for the creative thinking in the OL research field aimed at identifying a bridge between the random mixture of simple carbon-based molecules available on the early Earth and the simplest – but immensely complex – living entity that we can extrapolate from our biological knowledge. A large part of the scientific community focusing on the OL problem considers likely that new self-assembly mechanisms will be discovered, making the onset of biological complexity less indecipherable. This notion is supported by the fact that new mechanisms of self-assembly are continuously discovered in various areas of condensed matter science, hence making it conceivable that new revolutionary forms of molecular ordering will eventually be found. The shared feeling of this possibility creates expectation and will to experiment and speculate. The belief that relevant new knowledge is within reach promotes creativity. If any of these ingredients are missing (relevance, novelty, reachable success), scientific interest is easily lost. Lack of expectation damps interest. This is what is currently happening to the public fate of the investigations about the OL. Indeed, despite the rather large scientific community devoted to them, topics regarding OL are not raising large interest in the general public. This tendency can be related to various factors. For sure the lack of break-through discoveries has an important effect. More importantly, and at a more basic level, there is, in our opinion, a lack of expectation on what could be discovered. This is part of a more general loss of appeal of science in the western culture, affecting OL studies as well: could science (not technology!) convey concepts (not capabilities!) able to change our vision of life?

In this context it is hence of relevance to imagine what spectrum of ultimate scenarios could be possibly suggested by OL research. At one extreme we find the far-fetched, but

still conceptually possible, notion of demonstrating that the complexity of living beings is irreducible to the molecular mechanisms that are being studied. This concept, put forward by the supporter of Intelligent Design, appears, at the moment, lacking the necessary rational frame and evidences. At the other extreme, OL research could succeed in unraveling mechanisms leading to simple replicating and evolvable systems. This would indicate, de facto - or even de jure if new laws of complexity are found - that our Universe is structured so to favor the emergence of intelligence, a concept loaded of wonder for our very existence. Within this context, current scenarios, as well as the new concepts we have put forward in connection with DNA self-assembly, share the same basic cultural aim: reducing the “fantastic luck” implied by the fortunate assembly of functional molecules by introducing mechanisms imbedding a stronger degree of necessity. Self-assembly, however, relies on specific molecular properties. Indeed, the discovery (or exploration) of laws making the prebiotic events less dramatically improbable, points to combination of molecular properties and planetary conditions that is no less emotional. In a pool of simple, randomly synthesized molecules, some could respond to their environmental status exploiting specific properties to form structures in turn capable of replication and mutation. The very possibility that such molecular properties exist, if not fantastic, is at least certainly marvelous.

This introduction is followed by three more sections. In Section 2 we offer a brief description of the state of the art in the OL investigation and describe recent relevant contributions based on various forms of molecular self-assembly. In Section 3 we summarize the new evidence of self-assembling of nucleic acids and describe the elements that make it an interesting finding in the OL debate. In such a description we try to explicitly show what it is generally meant by “explaining” in the OL research. In Section 4 we address more general questions involving OL research, creativity and philosophical views: (i) the “essence” of life and OL research, (ii) insights into the public perception of OL research, and (iii) fantastic luck versus marvelous fine tuning.

2. Prebiotic scenarios and molecular self-assembly

2.1. A 500 million years wide gap

In the last decades, many attempts were undertaken (and some significant advances were obtained) to clarify some of the critical steps in life’s origin and evolution, such as the synthesis of first building blocks, the origin of RNA and DNA or the first cellular organization [1]. However, also given the difficulty to verify some of the environmental conditions on the early Earth, many of the issues are still highly debated [2], including the very definition of life. A definition that has attracted some consensus is the one proposed in 1994 by G. Joyce, and later adopted by NASA “life is a self-sustaining chemical system capable to Darwinian evolution”. This definition, however, leaves open some of the most crucial questions: are replication and mutability necessary features of life? Is a genetic code necessarily implied in an evolutionary process capable to produce beings of (in principle) unlimited complexity?

A more explicit definition would include the ability of autonomous replication and the possibility to keep and propagate information (and thus to take advantage on natural selection) [3]. Quite different approaches are also proposed. An interesting one is by A. Pross [4], who proposes to focus less on history and developmental process of the species and assign the notion of “life” to individual entities capable of goal-driven actions, a notion we will discuss further in Section 4.1.

Although the various definitions of life could in principle lead to identify “the” origin of life in different moments of Earth’s history, in practice any definition points to what happened in an interval of about 500 million years, around 4 billion years ago.

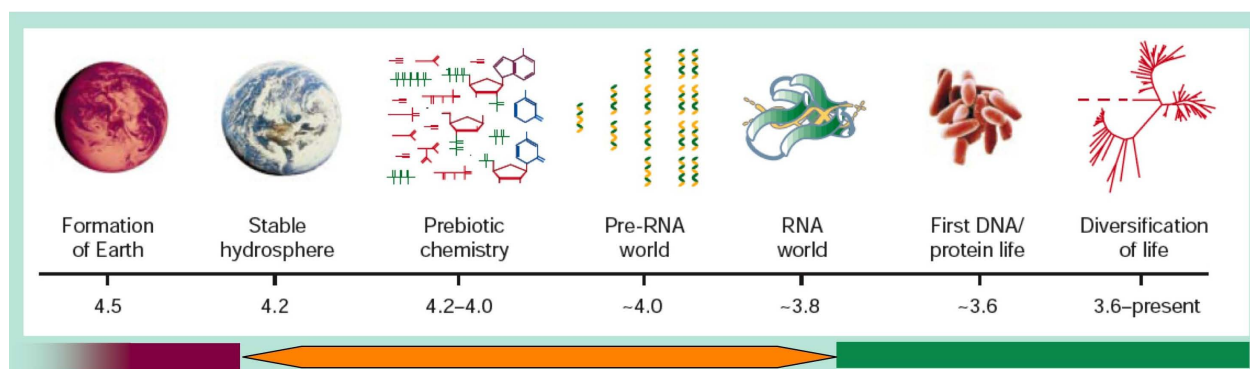


Figure 1 Timeline, expressed in units of billions of years, of Early life events, adapted from [5]. Colored bars indicate the era when the Earth could not have hosted life (purple), the era when life was certainly present (green), and the 500 million years interval (orange) when the origin of life took place.

In Figure 1, adapted from [5], the timeline of the main events regarding the origin of life is reported. The time axis can be thought as divided into two main sections, as indicated by the colors highlighting. The most recent portion (green) of the timeline encompasses the range of time in which we have paleontological evidence of life on our planet. At the other extreme, there is a time interval (purple) where no life could have been present because of the planet’s conditions. This leaves a gap where somehow the inanimate became animated. This is when crucial events took place and where OL research focuses its efforts. Possibly, the knowledge of the events in such time interval could even enable a better definition of life.

Scenarios about the events in the OL time interval are formulated (i) either moving forward in time on the basis of the planetary and chemical conditions of the early Earth, in an effort to understand how complexity could have possibly formed; or (ii) moving backward in time on the basis of life as it is known nowadays, with the aid of geological and paleontological evidence, in an effort to identify the simplest and more ancient forms of life.

Efforts to move “forward” in time need to be based on the necessarily partial knowledge of the early planetary conditions. The simplest organic compounds may have been present as soon as the Earth surface was filled enough by seas of water, the earliest evidence of crustal water being of about 4.3 Gyr ago. Hence in the range 4.3-4.0 Gyr ago we may assume

simple organic chemistry to have started being present, while, as detailed below, at 3.8-3.5 Gyr ago some primordial form of life was present. In between there is a gap of 500 million years that also includes the so-called “heavy late bombardment” at ca. 3.9 Gyr ago: a set of collisions probably bad enough to sterilize any existing form of life.

In 1952, Stanley Miller (under the direction of Harold Urey) tested the possibility of synthesizing organic compounds from inorganic precursors. Indeed, by applying for days an electric discharge to mixed vapors of H_2O , H_2 , CH_4 , NH_3 , at that time considered the most likely components of the early Earth atmosphere, he obtained various organic chemistry molecules, including aminoacids, nucleobases (adenine, guanine) and fatty acids, some of the building blocks for biotic molecules [6]. Later experiments by Miller himself, performed in more correct atmospheric conditions, did not produce such a large variety of simple compounds [7]. However, it has been recognized that a similar composition of simple organic compounds are found or produced in various conditions:

- i) the reducing gases, such as those originally assumed by Miller, can be found in localized environments such as volcanoes and vents, quite likely rather diffused on the early Earth [8];
- ii) the outcome of the Miller experiment when non-reducing gases (CO_2 , N_2) are used, very much depends on the presence of buffering compounds such as Fe_2+ ions, or pyrite, yielding, in some conditions, the same set of compounds as in the original experiment [9];
- iii) about the same organic molecules (amino acids and nucleobases) are found in carbonaceous meteorites (e.g. Murchison meteorite, Australia 1969) [10,11], indicating that conditions enabling the synthesis of these compounds could be found in the early Solar system;
- iv) nucleobases can be obtained in formamide in the presence of minerals acting as catalysts by simple thermal cycling [12, 13];
- v) a large variety of simple organic compounds are also obtained in aqueous solutions of ammonium cyanide (NH_4CN) at low temperature [14] and of hydrogen cyanide (HCN), heated or UV irradiated [15,16];
- vi) recently, a chemical pathway has been demonstrated for the stable formation of activated nucleotides from plausible prebiotic mixtures [17].

This set of results indicate that, even if we cannot really tell which way it happened, the early Earth could have been generally, or locally, rich in simple organic molecules, not dissimilar from the basic building blocks of nucleotides, peptides, hydrocarbons. This is why Miller’s intuition, even if based on a wrong assumption, turned out to be overall rather well confirmed. However, the availability of biomolecular precursors is far from indicating a path for the emergence of life. This becomes more evident by investigating the possible nature of the first and simplest forms of life.

Paelonthological evidence of life, such as fossilized bacteria, stromatolites, oxygen bearing

minerals, date at least to 3.5 Gyr ago, and maybe to 3.8 Gyr [18]. How could these first forms of life be organized? Investigations and speculations have focused on Last Universal Common Ancestor (LUCA), the mother cell of all living beings. Its existence is strongly suggested by the large set of molecular structures and processes shared by all living organisms, including the structure of RNA, DNA and proteins, the translation mechanisms, the use of ATP and many other biochemical structures and processes. It is currently believed that LUCA was a DNA and protein based organism with eukaryote-like RNA processing [19, 20]. Another interesting approach to the problem is to find the “minimal gene set” from today’s bacteria, i.e. the minimal ensemble of genes that enable a bacterium to survive in some standard conditions. Experiments indicate that a set of about 80 genes is indispensable for a bacterium to survive [21]. The set contains the code for proteins devoted to transcription, translation, DNA replication, metabolism, cell division. This finding implies a rather sophisticated cell life, way too sophisticated to have emerged through a discontinuous process. Can this cell organization be further simplified?

The most convincing answer so far conceived to this question leads to the so-called “RNA world”. The RNA molecule has a pervasive role in contemporary biology, especially with regard to the most fundamental and highly conserved cellular processes. It is involved as a primer in DNA replication and as a messenger that carries genetic information to the translation machinery. Even more interestingly, RNA is a crucial component of the ribosome – the actuator of the translation – whose core functional region is highly conserved throughout prokaryotes and eukaryotes [22]. Hence, if DNA were replaced by RNA, the transcription and translation processes could be replaced by a straight translation of the genetic code into proteins. Furthermore, it has been found that RNA may structure in “ribozymes”, i.e. RNA-made enzymes that perform various catalytic activities, such as assisting in RNA processing events and in functions related to the replication of viral genomes. This evidence makes it reasonable to imagine RNA molecules capable to replicate themselves. If we could find an RNA polymerase (i.e., an enzyme promoting polymerization) that was itself a ribozyme, then a simple ensemble of molecules might be capable of self-replication. The protein-nucleic acid world of contemporary biology could have emerged later in the course of evolution. Therefore, using the words of the Nobel laureate Walter Gilbert “one can contemplate an RNA world, containing only RNA molecules that serve to catalyze the synthesis of themselves” [23]. Accordingly, a self-replicating RNA molecule could have been the first “living” organism. Many were the scientists to contribute to this concept: the first time it appeared was by C. Woese, *The Genetic Code* (1967) [24], and in 1968, independently, F. Crick [25] and L. Orgel [26] also proposed that RNA preceded proteins. Investigations on the RNA world were later developed by G. Joyce and coworkers [5].

It thus seems conceivable that RNA was the first molecule having the capability to support life based on RNA genomes that are copied and maintained through the catalytic function of RNA itself, later replaced by the present machinery of DNA and proteins. Various

investigations were carried out to identify ribozymes with self-catalytic functions, the Holy Grail being a RNA replicase ribozyme. It appears evident, though, that sequences candidates for such a role could not be shorter than one hundred base pairs. Although this sequence length is short with respect to the length of the genetic sequences, it is actually impossible to imagine the formation of such a polymer on the basis of random chemistry. In fact, how the simple Miller-type molecules could have combined yielding life is the key question of the OL. In fact, how difficult is for current bioscientists to explain the formation of polynucleotides is implied by a quote by P.G. Luisi: “If a chemist is given all these compounds in any amount he wishes, he would be unable to make life. The fact that, until now, no oligopeptides or nucleotides have been detected in cosmic material may signify that these oligomers do not tend to form spontaneously.” [2]. In the same vein, C. de Duve, 1974 Nobel Prize in Medicine, in his book *Singularities - Landmarks on the pathways of life*, remarked [1]:

How RNA could possibly have emerged from the clutter without a “guiding hand” would baffle any chemist. It seems possible only by selection, a process that presupposes replication.[...] The need seems inescapable for some autocatalytic process such that each lengthening step favors subsequent lengthening. Only in this way could the enormous kinetic obstacle to chain elongation be surmounted. [...] Any invoked catalytic mechanism must accommodate the participation of a template, for there can have been no emergence of true RNA molecules without replication.

This is also echoed in a recent review article by another Nobel Prize in Medicine, Jack Szostak (Prize awarded in 2009) who writes that “the discovery of novel physical mechanisms is essential for a better understanding of how life could have began” [27].

2.2. The RNA-world. Information first scenario

Inspired by the notion that RNA is a molecule in principle capable of carrying and duplicating information and folding into chemically active secondary structures, many investigations have focused on developing ribozymes. Strategies of test-tube evolution have enabled obtaining several examples of ribozymes able to catalyze the template-directed joining of an oligonucleotide terminated 3'-hydroxyl to an oligonucleotide terminated 5'-triphosphate [28], and recently a natural ribozyme with similar properties (an intron from a cyanobacterium) has been reported [29]. However, RNA sequences of the order of 200bp have been found to enable ligation of up to 20 nucleobases [30,31]. Although a real auto-replicating ribozyme has not been found yet, and although many other problems should be solved to produce a convincing RNA replicase ribozyme scenario (such as the need of additional ribozymes to synthesize the nucleobases entering the ligation process), these findings are indeed impressive and keep the RNA world concept quite alive. Accepting the concept that Darwin-type evolution could operate at the simplified level of self-replicating RNA sequences, this would be the smallest molecular entity, so far conceived, capable to initiate life.

Despite these successes, the RNA-world view is disputed for various reasons. Firstly, although ribose, phosphate, purines and pyrimidines may have been all available in prebiotic

environment, their combination in RNA oligomers would have been a low yield synthesis because of the presence of the much larger amount of competing nucleotide analogues. Quoting again Luisi, “The proteins (or nucleic acids) existing on our Earth correspond to an infinitesimal part of the theoretically possible sequences – the ratio between possible and existing structures corresponds more or less to the ratio between the space of the universe and the space occupied by one hydrogen atom” [2]. Indeed, the nucleotides (and their analogues) may even have joined to form polymers, with a combinatorial mixture of 2'-5', 3'-5' and 5'-5'-phosphodiester linkages, a variable number of phosphates between the sugars, D and L-stereoisomers of the sugars, and assorted modifications of the sugars, phosphates and bases. The self-replication mechanism had somehow to accommodate these compositional differences and select the “right” nucleic acids [32]. In addition, only conveniently activated nucleotides can be ligated to a chain. Actually, the phosphorylation of mononucleotides and the synthesis of short oligomers was demonstrated in suitable extreme environmental conditions [33,34], but today the usual laboratory route is to use phosphorimidazolides of nucleosides or other activating groups [35] favoring polymerization, whose presence in prebiotic environment has not been proved.

Another class of objections raised against the RNA world hypothesis pertains to the activities of RNA catalysts, i.e. to the mechanisms that must have led to the emergence of specific, rather long (despite the relative fragility of long RNA polymers in aqueous solutions), active sequences over all possible sequences. Indeed, although it was demonstrated that oligo-Cs as short as four monomer units in length can serve as efficient templates for the synthesis of oligo-Gs from activated monomers [36], a RNA fragment length of 50-100 is assumed to be required for a good catalytic activity. However, 50-mers could be assembled in approximately 10^{30} different sequences, corresponding, if one molecule per sequence is considered, to about 3.5×10^7 kg RNA, a small fraction of which with catalytic functions. This impressive compositional redundancy makes the emergence of functional sequences quite a challenge.

In summary, if the building blocks of RNA were available in the prebiotic environment, if these combined to form polynucleotides, and if some of the polynucleotides began to self-replicate, then the RNA world may have emerged as the first form of life on Earth. Assuming its validity, the RNA-world somehow solves the “chicken or egg” problem between nucleic acids and proteins, but still leaves the following question unanswered: how did the first polynucleotides arise from monomers, without any enzyme, of whatever nature?

2.3. *Autocatalytic cycles. Metabolism first scenario*

In contrast to the “information first” scenario sketched so far, the other main theory, named “metabolism first”, claims that life arose from autocatalytic self-organizing chemical cycles [37].

For complex mixtures of reactants and products to move in the direction of life, a process of self-organization would be necessary. This process would enhance the concentration of certain components of the mixture, either at the expense of others, or by new synthesis from raw materials, with these changes driven by an external source of energy. Despite the absence of a genetic polymer, a transformed mixture of this type could be considered to hold hereditary information, which would be represented by the identity and concentration of its constituents (“compositional genome”). Evolution would be represented by changes in the composition of the system and in the reactions used to sustain it, in response to changes in the surrounding environment. Growth of the system would take place through the acquisition or synthesis of additional quantities of the key components, and reproduction would occur when physical forces split the enlarged system into two or more fragments.

Unfortunately, no plausible self-sustaining chemical cycles have been found so far, and thus even the proof of principle is still missing. Therefore, in the current absence of any other reasonable precursor, the RNA model represents a system that allows us to explore essential aspects of the emergence of a polymeric, genetic system without the requirement of a complex metabolism.

3. The self-assembly of nucleic acids

3.1. *Liquid crystals made of oligomers of DNA and RNA*

We have recently observed a previously unnoticed self-structuring behavior of fragments of DNA [38,39,40,41]. This discovery may impact the current views of prebiotic events. Specifically, we have found that mixtures of complementary and not complementary very short strands (≥ 6 base pairs) of DNA or RNA display the following behavior, sketched in Figure 2:

- i) mutually complementary sequences hybridize forming fragments of double helices, as expected (Figure 2A);
- ii) such short segments of double helix aggregate end-to-end into longer helices, the length of the aggregate being larger for more concentrated solutions. The mechanism for the aggregation is the “base-stacking” attraction, quite known to act within double helices but seldom considered as a form of inter-double-strands interaction [42, 43] (Figure 2B);
- iii) these necklaces of reversibly aggregated fragments mutually order into liquid crystalline phases. At a lower concentration, the duplexes align in a common direction which forms itself a supramolecular helix (“cholesteric” phase), either right-handed or left-handed depending on the specific sequence [40]. At higher concentration they align, forming, in a plane perpendicular to the linear aggregates, an hexagonal lattice of “columns” free of slide with respect to each other (“columnar” phase). The geometry of the columnar phase is described in Figure 2C. These forms of ordering are truly long range, and yield micron sized domains of optically anisotropic fluid, easily detectable through optical



polarized microscopy, such as shown in the inset of Figure 2C and those of Figure 3;

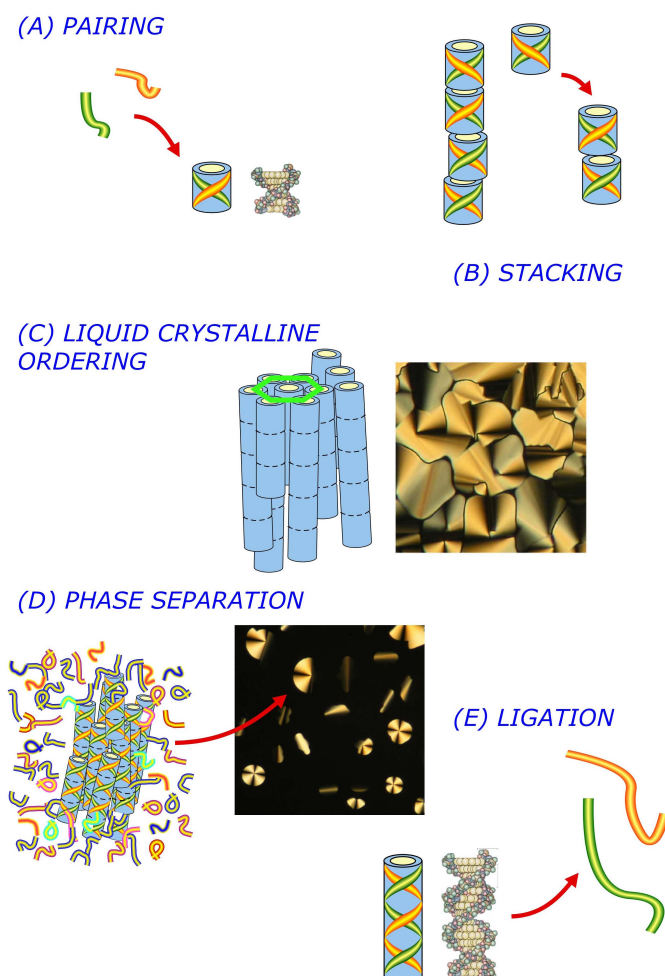


Figure 2 Motifs of self-assembly observed in solutions of short DNA and RNA oligomers. (A) The oligomers, if mutually complementary to a sufficient degree, aggregate, arranging as double helices. (B) DNA and RNA duplexes, when in concentrated solutions, form linear aggregates held together by stacking forces. (C) Such aggregates are characterized by large enough axial ratio to order in liquid crystalline phases, such as the "columnar" phases, where the columns are arranged with hexagonal symmetry as in the drawing. This arrangement is easily recognized in polarized optical microscopy through the appearance of textures as the one shown in the picture, representing a portion of $50 \times 50 \mu\text{m}$ of a thin cell hosting a oligomeric DNA duplex solution. (D) When the solution contains both single strands (unpaired oligomers) and duplexes (paired in double helices), the system spontaneously phase separate into liquid crystalline domains, rich in duplexes, and isotropic fluid, rich in single strands. The drawing pictures a set of ordered columns (made of rigid aggregated duplexes), surrounded by the disordered and flexible single strands. The picture shows the phase separation observed in mixtures of DNA oligomers: the dark portion correspond to the isotropic fluid of single strands while the colored part are the liquid crystalline domains of ordered double helices. (E) The aggregation in columns of duplexes is a spontaneous template favoring chemical ligation between the oligomers. Hence, the ordered stacking of DNA and RNA duplexes may act to promote their spontaneous chemical elongation.

iv) when double strands are mixed with single strands (i.e. sequences lacking their complementary one), duplexes phase separate, segregating from the mixture and forming fluid droplets of highly concentrated double strands organized in liquid crystals, coexisting with a second fluid rich in unpaired strands (Figure 2D). The dark background in the polarized microscope picture in Figure 2D is given by the isotropic fluid of single DNA strands. The bright features correspond to droplets of ordered DNA strands. The same behavior of duplex condensation is found when fragments of double helices are mixed with other polymers in solution, such as polyethyleneglycol (PEG). This is thus a mechanism favoring the condensation and the purification of

DNA duplexes;

- v) this self-assembly mechanism is found to be active even in solutions of sequences that are not fully complementary. A first extension involves sequences that pair in duplexes with overhangs, i.e. short tails of unpaired oligonucleotides. If the overhangs are chosen so to be mutually complementary, duplexes aggregate through the pairing of the tails, yielding the same liquid crystal phases in approximately the same conditions as for the fully complementary duplexes. Long range ordering is however also found when the overhangs are random, a situation that corresponds to solution of duplexes having a large variety of overhang sequences. In these cases, aggregation and liquid crystal phases are found (with some dependence on the length of the overhangs) because on average, the encounters between random sequences leads to non-zero association energy [44]. A further surprising extension was finding that fully random sequences can still lead to liquid crystal ordering. This has been observed only for sequences whose length is between 16 and 30 bases. Following various evidences, this effect appears as a consequence of the statistics of the duplexes that form in such a solution. Such a population of duplexes – typically rich in mismatches – is such to provide, through overhang interaction, aggregation and macroscopic ordering.

It is interesting to note that this cascade of self-assembly is triggered by the Watson-Crick pairing events. A somehow similar phenomenon is known in solutions containing Guanosine, one of the four nucleobases (while not in solutions of the other three). Guanosine has only a weak propensity to stack as a mono-nucleotide. However, the geometry of the molecule enables Guanosine to form flat quadruplets, connected side-to-side by H bonding.

Aggregation in quadruplets enormously reduces the water solubility of the molecules because of the enlarged hydrophobic surface. Quadruplets hence stack in column. Here again, pairing through H-bonding triggers stacking. These examples convey the interesting notion that self-assembling may induce further self-assembly, in a cascade that is quite difficult to predict, and even more difficult to exploit through molecular design.

We have proposed that the self-assembly of oligomeric double helices depicted in Figure 2A-D could be a new route for the prebiotic synthesis of polynucleotides, more realistic than most of the current OL research as for how the long biological homopolymeric chains could have formed in the prebiotic Earth [5]. Indeed, this rich staged form of self organization, with its phase separation, end-to-end stacking and liquid-crystalline ordering of helices acts to promote complementarity by condensing duplexes and positioning the oligomer strands close to each other in the exact geometric arrangement that best favors chemical ligation, i.e. with contacting terminal bases and possibly with bases oriented at the mutual angle that provides continuity to the phosphor chains across the linear aggregate. Hence, if conditions are such to favor chemical ligation between contacting phosphate and ribose group (Figure 2E), the packing implied in the self-organization could not only provide a very favorable

spatial arrangement of the molecules, but also a feedback mechanism for further elongation and selection: a chemical growth of the ordered strands leads, upon thermally cycling, to a better liquid crystal arrangement in which the longest oligos are the first to condense, a condition that favors further chemical lengthening.

Whatever theory is thought to be the most adequate, the critical step for the dawn of RNA (or DNA) as the information carrier lies in its elongation from single nucleotides, or at best oligomers formed by random chemical ligation, to the long biopolymers our lives are based on. By using the words of a scientist that has devoted most of his energy in investigation the RNA world scenario [5]: “The chief obstacle to understanding the origin of RNA-based life is identifying a plausible mechanism for overcoming the clutter wrought by prebiotic chemistry.” G.F. Joyce.

In this vein, we briefly discuss below two of the conditions that may have helped in promoting this crucial step of molecular elongation: increment of the local concentration and selection of reactants and template.

3.2. Relevance for OL scenarios: local concentration and molecular selection

To enable encounters and reactions between the simple molecules available on the early Earth, some kind of mechanism was certainly at play, especially since there is evidence that prebiotic oceans were as dilute as contemporary ones.

Life is now organized into cells, very complex “worlds” basically separating genetic material (and various degrees of organelles and molecular machineries) from the outside through selectively permeable lipid membranes. The most natural and conservative approach would appear to imagine some simple proto-cells, combinations of RNA and surfactants, achieving the same results and being able to replicate [3]. This possibility is made more interesting by the fact that some surfactant micelles and vesicles were found to spontaneously split (and thus “self-replicate”) under appropriate conditions [45,46]; furthermore, primitive membranes were demonstrated to allow the entrance of single nucleotides while retaining oligomers, i.e. the result of ligation [47]. Although similar phenomena had certainly to occur for the birth of the first cell to take place, it appears unlikely that this was the real driving force for the original RNA segregation and elongation, since this would imply a locking mechanism between the duplication of vesicles and the evolution of RNA, which would in turn imply sophisticated machineries reflecting, through molecular synthesis, the RNA sequence to the behavior of the vesicles.

Hydrothermal marine environments, characterized by heat currents flowing through porous minerals, could have played a role in the development of life, providing a heat source, minerals in solution and fluctuating conditions. A spontaneously increased concentration of RNA strands could have been promoted by convection and thermo-diffusion [48].

In a rough estimate, a 10^6 -fold accumulation is required for small protobiomolecules to interact. Also surfaces and structured porous minerals could have promoted, by preferential adsorption, an increased surface concentration of prebiotic molecules and act at the same time as catalyzers, as discussed in the next section.

All these mechanisms lack however the necessary capacity of selective concentration. Enclosure into vesicles and thermo-diffusion could have promoted local enhancement of molecular species, but without relevant selectivity among the huge ensemble of molecular variants. Surface adsorption is more specific, but it is hard to imagine an adhesion process that would be strong enough to induce local crowding but weak enough to enable mixing, collisions, interactions and all the molecular events necessary to the formation of complexity.

Our observation of the capacity of duplexed RNA fragments to condensate out from a mixture containing unpaired sequences and other flexible polymers [37, 38] adds a new interesting concept to this set. Short RNA (and DNA) duplexes spontaneously segregate from richer molecular mixture mainly because the hybridization process strongly modifies the molecular property. Single strands are highly flexible, while duplexes are rigid. Moreover, single strands are mutually repulsive because of the electric charges they bear, while duplexes arrange so to expose, at their ends, the hydrophobic surfaces of the paired nucleobases that can hence interact with each other attractively. There is therefore a subtle correlation between the molecular structure enabling duplexing and segregation of the duplexes. Certainly, the phase separation of the well-formed helices needs a significant concentration to start with. Also, the robustness of the phenomenon and its sensitivity to unpaired nucleotides need to be further tested, as currently in progress. However, despite the still rather stringent conditions in which the phenomenon is observed, our findings convey the concept of a new possibility: the chemical structure can – through self-association – induce self-purification and enhance concentration.

3.3. *Relevance for OL scenarios: template*

High concentration of oligonucleotides alone is not sufficient to sustain polymerization. Inspiration comes from the fact that most of the known enzymes work by geometrical and physical constraint, i.e. by keeping close together active groups and thus enhancing reaction rates. In analogy, scientists in the field regard as necessary some form of template mechanism to favor ligation of nucleotides. In the words of C. De Duve [1]:

The need seems inescapable for some autocatalytic process such that each lengthening step favors subsequent lengthening [...] Only in this way could the enormous kinetic obstacle to chain elongation be surmounted. [...] any invoked catalytic mechanism must accommodate the participation of a template, for there can have been no emergence of true RNA molecules without replication.

The appeal of RNA as the first self-replicating molecule relies on the fact that, by definition, it would be capable of acting autocatalytically for its own synthesis and, at the same time,

such autocatalytic molecule would act as a template to bind the precursors by non-covalent forces and organize them in such a way that the reactive groups come in close proximity. Studies with activated trimers and hexamers showed that template autocatalysis can only occur if the sequences of both trimers match the sequence of the hexamer according to the Watson-Crick base-pairing rules. They also showed that the condensation reactions are predominantly controlled by the stacking of nucleic acid bases flanking the newly formed internucleotide link [49]. As already said, however, we are left with a new “chicken or egg” problem: how did the first templating RNA oligomer arise without a template? Bulk condensation polymerization reactions are usually thermodynamically driven towards hydrolysis in dilute aqueous solutions. Therefore, besides high concentration, a surface-promoted mechanism is required to enhance the polymerization rates. Some mineral surfaces have been proposed as good templates for nucleotide polymerization. The most credited candidate is montmorillonite, a clay mineral with a layered structure. Reversible hydration or solvation of the cations cause the layers to expand, favoring the entrance of certain molecules [50]. A number of experiments verified the binding of mononucleotides onto the montmorillonite surface or inside its layers and its ability to promote the formation of the phosphodiester bond (in suitably activated monomers) and thus the elongation of nucleotide polymers [51, 34, 52]. Interestingly, montmorillonite was also reported to favor the homo-chiral selection of nucleotides [53], another critical step in the development of longer molecules [1].

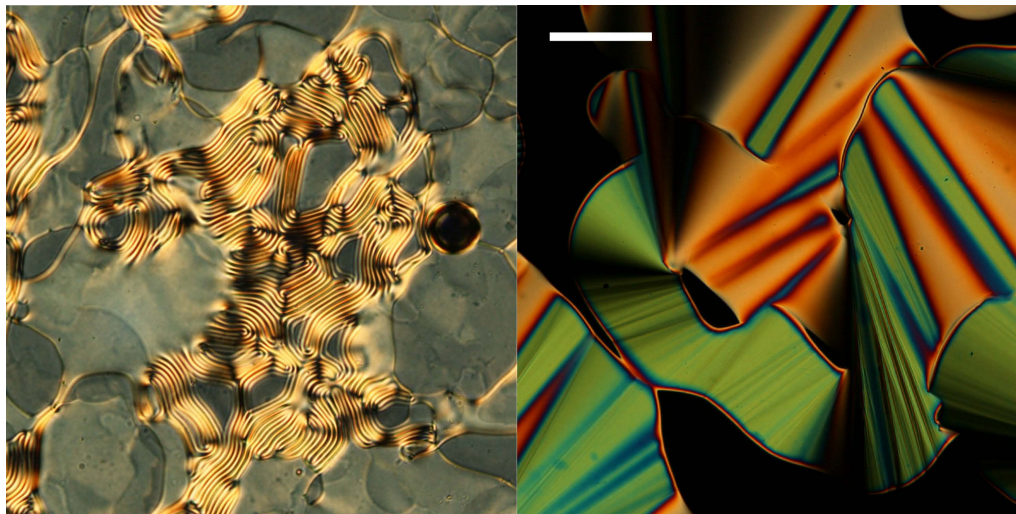


Figure 3 Photographs taken at the optical polarized microscope. The pictures report the light transmitted through a thin cell (10 μm) containing the DNA solution, placed between crossed polarizers. Colors indicate the quality and orientation of the ordered structures. The white bar corresponds to 20 μm . The picture on the left is taken at a lower concentration (20% vol. ca.) and its textures are characteristic of a “cholesteric” liquid crystal phase. In this phase the aggregated columns arrange into super-helices, whose pitch is given by the distance between the lines visible in the picture. The picture on the right is taken at a larger concentration (50% vol. ca.) and its textures are characteristic of a columnar phase (colored) coexisting with an isotropic phase (black).

However, in clays the catalytic surface is a liquid-solid interface, and the lack of fluidity in this interface could be poorly compatible with an efficient surface diffusion [54]. Such surfaces also lack the flexibility which is found in present-day enzymes, and which is known to be crucial to catalysis. Accordingly, some other mechanisms have been proposed,

involving liquid or “soft” phases. Oparin [55] suggested that prebiotic polymerization reactions took place in a heterogeneous, coacervated system, rather than in the bulk of a homogeneous phase. Coacervation, a liquid-liquid phase separation, was considered as an essential concentrating process by which mixtures of randomly formed prebiotic polymers initially in dilute solutions were condensed into concentrated assemblies. Although naive (he thought that coacervated droplets directly lead to cells), Oparin’s theory introduced for the first time the idea that a physical phase separation process could lead at least to locally enhanced concentration of nucleotides and thus more favorable elongation.

The proposal of the catalytic role of a liquid-liquid interface, namely between an oil slick and salty water, was put forward by Lars Onsager [56], and the renaturation process of DNA oligomers is found to be enhanced at the interface between phenol and water [53]. Other proposed systems acting as oligomerization template are gel matrices [57] or eutectic ice-water mixtures [58, 59], but none of these theories is supported by unambiguous experiments.

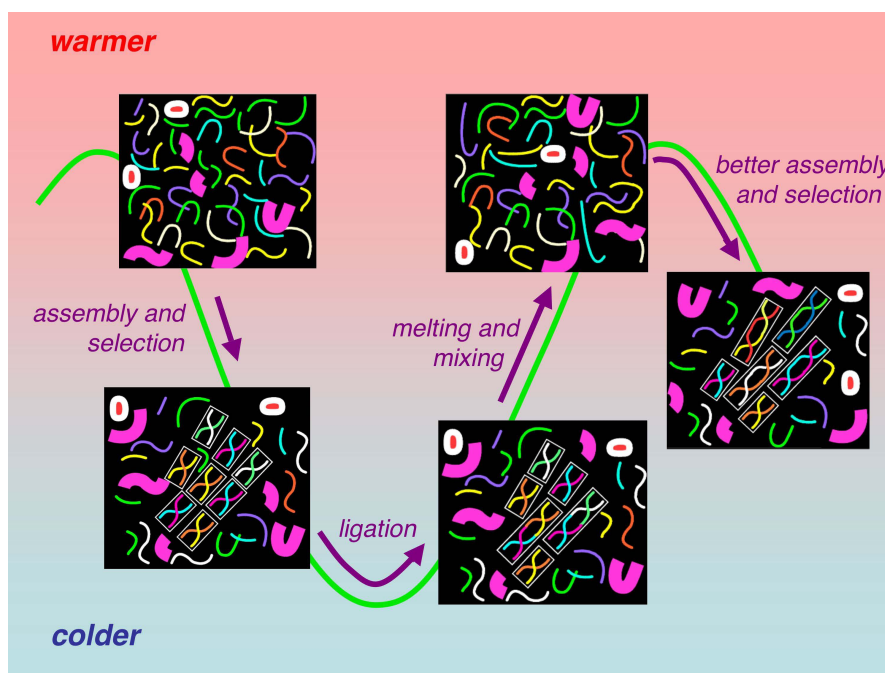


Figure 4 Schematic description of the possible mechanism in which the self-associative properties of RNA oligomers are shown to promote the polymerization of RNA. We imagine a solution containing RNA oligomers as well as other molecules. Upon cooling, the self-association of RNA lead to the formation of liquid crystal domains, a condition in turn favoring ligation of the oligomers into longer molecular chains. As the temperature rises again, liquid crystal domains and double helices melt. The solution is now richer in longer RNA molecules. Hence, upon cooling again, the formation of liquid crystal domains will be easier to obtain (at higher temperature and lower concentration) and it will be more selective as the possible spurious molecules dissolved in them. This will promote further elongation. The cycle may continue until the RNA length is large enough to yield the first working ribozyme.

Recently , RNA monomers were found to polymerize when subjected to dehydration cycles at moderate temperature, in a mixture with simple lipids [60]. At high concentrations, the lipids organize in lamellar and possibly hexagonal phases, thus providing a template for the linear elongation of the RNA strand. Again, the observed spontaneous LC ordering of short RNA strands emerges, in our opinion, as the most simple and non-redundant template mechanism: without relying on additional ingredients, it provides a self-templating structure.

The LC matrix provides a flexible template, possibly favoring chemical ligation, and thus promoting an auto-selective process, since longer helices can more easily fit in the aligned environment.

3.4. *Are we explaining anything?*

The self-association of oligomers and formation of ordering could have played a role in its selection and emergence from the prebiotic molecular clutter via the promotion of molecular elongation through template-driven ligation of adjacent nucleobases. In other words, self-assembly could have had a role in the emergence of nucleic acids as informational biopolymers. The scenario we propose is summarized through a cartoon in Figure 4. The scenario starts from solutions containing a sent of random chemical, including short oligomers of nucleic acids. In such a pool multiple thermal cycling between warmer to colder temperatures could have occurred. As the temperature T is lowered liquid crystalline domains of duplexed oligomers are formed. In this way the duplexes are selected out of the clutter, concentrated and positioned so to promote ligation. As T is raised, duplexes unbind, and the solution is remixed. However, the fluid has changed since some fraction of the oligomeric duplexes have chemically connected. Upon lowering T again, the longest duplex-forming oligomers will order and segregate first, their melting T being larger and their threshold for liquid crystallization lower. Moreover, as the length grows, the liquid crystallites are better defined and their solubility of the different chemical species decreases. In this way, the longest oligomers will have a larger chance to elongate further, thus constituting a positive feedback pushing toward polymerization.

Even if this picture is correct, however, it would constitute not more than a ring in a chain that is still, for the largest part, missing. Indeed, it is not clear how the nucleic acids oligomers could have formed up to the minimum length where we observed liquid crystallization, i.e. 6 base pairs. Furthermore, even if the elongation process would succeed in yielding polymers long enough to have the potential to structure into ribozymes (i.e. of length of the order of 100 base pairs), the compositional abundance of chains of such length is so enormous ($\sim 4^{100}$) to make the identification of any specific structure apparently impossible. Certainly, so far, “searches of quadrillions of randomly generated RNA sequences have failed to yield a spontaneous RNA replicator” [61]. Furthermore, to be a quasi-specie, the RNA replicator and the other ribozymes necessary to produce the building blocks need to be coupled and to an environment that holds them together, such as a lipid vesicle. But in this case, all parts in such a proto-cell must be coupled: an efficient replicator must lead to a more successful quasi-cell and not be suffocated, caged or sequestered by an indifferent membrane.

But even by restricting our will to understand the OL to the specific problem of the elongation of RNA oligomers, does the self-association of RNA explain anything? The formation of the right polymers is a purely statistical matter. Hence there is no real objection to its appearance other than the extreme improbability of its occurrence. Self-association

of nucleic acids makes this process less improbable, at the same time shifting its focus to the molecular features making the process possible. It makes the chemical ligation between nucleobases “less improbable” since it relies on the capacity of nucleobases to stack to each other. While we will further comment on this in the last section of this article, we summarize here which are the molecular properties essential to the self-assembly. RNA and DNA are flexible homo/heteropolymers where the heterogeneity (i.e. the existence of 4 different nucleobases alternated in the chain) does not significantly modulate the chemical/physical properties along the polymer. The nucleobases are capable of generic stacking (hydrophobicity) and specific pairing (H-bonding) interactions, so to form paired strands. It is worth noticing that, given the pairing capability, the alternation of the 4 bases and the presence of electric charges along the phosphate chains minimizes the formation of aggregates other than sequence-matching double helices. The duplexes are much more rigid and are terminated either by neatly paired endings (“blunt ends”) or by one or a few overhanging bases. Mixtures of rigid aggregates and of smaller and/or more flexible molecules are typically unstable and tend to phase separate because of “depletion-type” entropic forces: the global phase space is increased when solutes of sufficiently dissimilar steric properties are geometrically separated in the solution. The duplex endings, whether blunt ended or with overhangs, can interact with the endings of other duplexes through stacking and pairing forces to form linear aggregates. The aspect ratio of the aggregates, increasing as the aggregate gathers more duplexes, favors the formation of ordered, liquid crystalline aggregates, in turn stabilizing the aggregate. Liquid crystalline ordering and linear aggregation of duplexed oligomers into long chains are mutually strengthened, yielding a marginally stable order. The weakness of the self-association of this molecular species is crucial since its easy disruption by thermal cycling gives way to a better selection of the longest, best paired duplexes as T is lowered again, a process at the heart of the positive feedback motive for the elongation of this self-associating polymer.

Hence, the proposed picture points to the existence of molecules capable of an amazing cascade of pairing, stacking, and self-association in ordered structures prone to chemical ligation, a set of properties that enabled self replication and hence life information storage. Certainly some of these properties were crucial in the selection of nucleic acids as the carrier of genetic information. The very existence of molecules embodying all these properties is not obviously deducible from the basic knowledge of organic chemistry. Furthermore, if this was indeed the pathway for the emergence of life, it was necessary that these properties were not shared by many other molecular species, so that RNA could have emerged without too strong a competition from molecules sharing similar properties. Hence, the proposed scenario points to a delicate fine-tuning of factors that we could name “marvelous” because (i) there is, rather surprisingly, a molecular species that shows all of them and (ii) because they are so delicately balanced to be extremely rare within the vast realm of molecular species. Both factors are certainly, but subtly, necessarily implied by the basic structure of matter (electron charge, proton mass etc.) and hence related to the basic architecture of the Universe.

4. Investigating the origin of life: science and beyond

4.1 *Fantastic luck and marvelous fine-tuning as reference concepts in biological sciences*

Although “fantastic luck” scenarios are not forbidden by natural laws, they appear increasingly unlikely and hence “unacceptable” to the sensitivity of the scientists. As we have seen, the direction taken by the OL research is to propose scenarios where the “fantastic luck” is reduced, and replaced by a stronger degree of necessity. How far this could go, how much our existence can instead be viewed as necessary, woven in the deep structure of Nature, is a question that has always interested scientists. Its answer has reflected the changing sensitivity of the different cultural periods.

Before Darwin many leading biologists have devoted their research in the study of the recurrent forms of the organic world, such as the forms of leaves and the pentadactyl design of the vertebrate limb [62]. There was a common belief in the existence of a finite set of “natural laws” or “construction rules” defining the major characteristic of the biological forms, in analogy to the rules accounting for the construction of the periodic table of elements in chemistry and the laws of crystallography. At that time, the crystal was one of the most popular metaphors for organic forms. The formation of cells was seen as a kind of crystallization process, and organisms as an aggregate of such crystals [63]. A small number of basic pattern and symmetry rules allows the construction of many different crystals with different properties. Similarly, some not fully understood set of laws would have governed the diversity of the organic forms. According to this view, any possible form of life in the universe should necessarily have characteristics similar to the organism on Earth.

In post-Darwinian biology the necessity of the natural law was replaced by the contingency of natural selection. During the course of evolution, organic forms were now viewed as put together piece-by-piece by naturally selecting the best biological function among those emerged by chance. Organisms were now more similar to an artifact such as a watch rather than a crystal. Analogously to a well-made and sophisticated watch, the assemblage of organisms was primarily defined by their function, through the continuous process of random changes and selection of the best performance. In principle, there are unlimited ways to build a watch and similarly the known forms of life must be just a tiny fraction of the infinite possible forms.

In recent years, an increasing role is being attributed to possible intrinsic evolutionary “constraints” imposed by the laws of physics and chemistry and presumably by biology itself. Many examples of convergence evolution of both complex organs such as the eye and sophisticated molecular machineries such as the retinal-opsin protein system to transduce light into cellular signaling, strongly suggest the existence of a limited number of favorite evolutionary routes. In the same vein, a narrow set of lawful natural forms has been proposed to account for the unexpected finding of a limited number of naturally occurring protein

structures, which results from crystallographic studies. Despite the fact that the number of conceivable molecular conformations is astronomically large, the number of solved different protein structures tends to saturate to less than a thousand. Such structures seem to represent preferred arrangements determined by intrinsic “organizational laws” of matter. This observation suggests a change of point of view: natural polypeptide chains can be thought of just as a particular chemical realization satisfying the allowed organizational rules of matter. In other words, among all “spontaneous” chemical compounds, only those able to fit the assemblages restricted by fundamental physical constraints could have been selected by nature. It is emblematic that this frame of thought has lead scientists to use expressions such as “Platonic forms” [64,65] to indicate the geometries really accessible by the immense possible variety of amino acid sequences of proteins.

In some analogous way, scientists in the OL research field are currently aiming to uncover fundamental “laws of forms”. The growing body of knowledge in molecular self-assembly is currently offering new “forms” for the structuring of matter: nowadays, the emergence of life cannot be conceived without referring to bilayers, vesicles, liquid crystals, fractal aggregates.

4.2 The public perception of the OL: what could be expected from science?

While the feeling of new pivotal discoveries motivates the scientific community, we wish to comment on the public perception of the OL problem. An idea of the perception of the OL research in a non-specialist audience can be retrieved through a survey of the results obtained by searching the topic “origin of life” in the World Wide Web and disregarding sites directly connected with research institutions. What appears is a prevalence of strongly polarized visions on this topic, that use the OL theories in support to religious or ideological visions of the world. For example, in “Why abiogenesis is impossible?”¹ a creationist group denies the possibility of abiogenesis sustaining that Miller experiments is a blind alley as “demonstrated” by the impossibility in the last fifty years to find a “self-assembly” process that randomly links aminoacids to form more complex structures. Their conclusions is that “abiogenesis is only one area of research which illustrates that the naturalistic origin of life hypothesis has become less and less probable as molecular biology has progressed, and is now at the point that its plausibility appears outside the realm of probability”[65]. In other words the lack of results in the “...experiments [of Miller and others] have done much more to show that abiogenesis is not possible on Earth than to indicate how it could be possible”².

A similar attitude can be found in websites with a different religious background, being them Christian³ or Islamic⁴. Conversely, activists of atheism⁵ proclaim that the recent results of scientific research about the OL are proofs of mechanicism, in turn supporting

1 <http://www.trueorigin.org/abio.asp>

2 <http://www.trueorigin.org/abio.asp>

3 <http://www.answersingenesis.org/tj/v18/i2/abiogenesis.asp>

4 <http://www.islamfortoday.com/emerick16.htm>

5 <http://www.infidels.org>

the notions of the non-existence of God. In both cases the actual scientific debate has been cut off. For example, in the “ConservativePedia”⁶, a politically-oriented version of Wikipedia that dedicate several pages to evolution and origin of life, the expectation level of scientific community is summarized by a sentence of Lee Strobel, from the book *A case for faith*: “The optimism of the 1950’s is gone. The mood at the 1999 International Conference on Origin of Life was described as grim-full of frustration, pessimism and desperation.” The expectation of turning-point discoveries that actually stimulate scientific research has been removed and substituted by well established ideological positions: “Despite repeated attempts under every reproducible circumstance, atheistic scientists have been unable to reproduce a reasonable method for the origin of life without a creator, nor do they have a clear understanding of the chemistry involved”⁵. Quite interestingly, both atheism activists and creationists agree on the fact that the scientific research on the OL and religious beliefs are intrinsically irreconcilable. This is, of course, in remarkable disagreement with the official position of the Catholic Church [66] and the opinion of authoritative scientists [67].

Also, OL has been the topic of a number of articles in newspapers. However, its impact is certainly much less than more ethical aspects of life, such as for instance, “the quality of life” or “the end of life”. It is also considered less relevant than somehow similar questions such as the origin of the Universe. Google searches, November 2011, indicate that the “origin of the life” and related topics (excluding search connected with explicit religious reference as “creation”) are reported on the Web about 5 times less than “Big Bang” and related topics. Interestingly the same search performed in 2009 have conveyed a larger discrepancy, with “origin the life” being 30 times less cited than “big bang”, suggesting that the implications of the on-going scientific research on OL is slowly infiltrating the common perception. Furthermore it has to be noticed that recent scientific achievements as the creation of the first artificial cell [68] and the discovery of a skeleton of *Australopithecus Sediba*[69], even though not directly related to the OL, have captured general attention and turn it toward a deeper questioning about who we are and what is our origin. A further catalytic event that focuses public interest on these specific questions has been the 150th anniversary of the publication of *The origin of the species* (2008) and the 200th anniversary of its author’s birth (Charles Darwin, 1809-1882), which has generated a widespread debate ranging from evolution to other connected problems as OL. In 2009 a British movie interestingly entitled “Creation” has portrayed the life of Charles Darwin and the struggle between him and his religious wife about the consequences of his theories. In 2011 the awarded movie of Terrence Malick, “The Tree of Life”, has represented with evocative poetry the connection between the origin of the first form of bacteria and nowadays life.

In the last years scientists in the OL field have also published a number of books for general audience [1,70,71,72] discussing the recent scientific discoveries and their implications

6 http://www.conservapedia.com/Origin_of_life

on the vision of the world, often attacking the creationist approaches and defending their discoveries and theories. These new publications reflect the renewed expectation of the scientific community of an imminent discovery which will be significant not only for specialists but will have far-reaching consequences.

It is also interesting to inspect how the topic is presented in educational programs. We have sampled this issue by analyzing Italian middle school textbooks. We inspected eight books that cover about 95% of the middle school market. We generally found correct accounts, necessarily minimal given the level of the school, but (i) without any emphasis on the fact that this is a research topic still open for new solutions and (ii) totally lacking the suggestion and encouragement of expectation. There is generally the acknowledgment that the origin of life is an “unsolved problem”, followed by the description of the Miller experiment and a set of statements about what could have happened^{7,8}. In none there is a remark about the implications that this crucial moment in the history of our planet – and possibly of the entire Universe – has on our understanding of ourselves as living beings. Certainly this attitude reflects the fact that (alas) for many it appears easier to teach science as a body of established knowledge rather than as an ongoing research. This attitude, however, also express a general perception of the topic. Questions such as “are we extremely lucky? Is our presence implied by the basic laws of physics?” would appear very appropriate and an easy way to foster attention and interest. They would require, though, the recognition that science can still offer relevant findings for the human culture and for a better understanding of the mystery of our being.

We speculate that a rather profound reason for the current lack of interest in the topic of the origin of life is the loss of expectation of new relevant knowledge, of hope for new understandings about the essence of life. In part this is certainly due to the lack of breakthrough discoveries in the field. However, as this article witnesses, some relevant findings have been produced. The reason for the loss of interest is, in our opinion, rooted in the perception

- 7 In the Chapter “The birth of life” of the school textbook “Universo Scienze C - Biologia: i viventi”, by Flaccavento and Romano, published by Fabbri Editori, besides the description of the Miller experiment, here omitted, we could find (our translation from Italian): “UV radiation and the electric discharges from lightnings induced chemical reactions among atoms and molecules dissolved in the primeval soup. In this way more complex molecules were generated, such as the aminoacids, the simplest organic molecules present in the living organisms. Sugars and proteins were also later formed. Finally, more complex structures were formed, the coacervates. Around such structures a membrane was formed that separated it from the external world, making them capable of accomplishing chemical reactions and to feed themselves with the organic chemistry in the surrounding environment. The most complex coacervate droplets specialized to the point of being able to reproduce: within them, in fact, nucleic acids were formed, responsible for cellular reproduction. This enabled coacervate to duplicate splitting in two parts: the first prokaryotic and heterotrophic cells were formed and this marked the origin of life, with the appearance of the first being capable of reproduction. [...] It is thus conceivable to imagine that, on the basis of simple compounds as water, ammonia, methane, hydrogen, 4 billion years ago the first organic molecules were formed and from them ... life”.
- 8 The textbook “Linea Scienze” by Leopardi and Gariboldi, published by Garzanti, concludes in this way the section devoted to the origin of life (our translation from Italian): “In the “primeval soup” the first molecules perhaps reacted among themselves, organizing into more complex structures called “coacervate” droplets, that is specific aggregates of proteins and other compounds surrounded by a water film. In a later time, coacervates would have merged into larger droplets that include smaller droplets (complex coacervate droplets). The transformation of the first aggregates of organic compounds into cells took place probably through the spontaneous formation of protein-based or lipid-based microspheres that could segregate inside various compounds. At a later time, other fundamental steps were required, such as the formation of a cell membrane, a specific internal organization, the appearance of molecules capable to store and use energy, the formation of molecules capable of storing and transmitting information (DNA), the formation of enzymes to carry out the chemical functions described above.”

of life that the continuous progress in biology has provoked. Although the cellular life is still for the largest part unknown, the overall understanding is that we basically know all what matters in the functioning of the simplest life forms, such as bacteria. The first forms of life – presumably even simpler than bacteria – are now viewed as “nothing but” biochemical machineries, where the distinction between organic and inorganic is faint. Hence, explaining biochemical machinery with, for example, self-assembling processes among carbon-based molecules, appears neither particularly revolutionary nor significant for the understanding of our own existence. This perception is quite different from the popular imaginary of the 50’s and 60’s about the simplest life form. Microbes were considered, somehow, a whole new world to be explored, where a large variety of mysterious beings, possibly gifted of unexpected capacities, could have been found. The consideration of the biological life has since then changed. Already in 1970, the Nobel Prize winner Francois Jacobs (Nobel Prize in Medicine awarded in 1965) in his book *La logique du vivant* wrote: “In our laboratories we don’t investigate life any longer. No longer we try to define the boundaries of life. We only analyze living systems, their structures, their functions, their history”. Microbes are nothing but microbes, ensembles of sophisticatedly coupled genes, proteins, enzymes. Hence, explaining the origin of bacteria appears less relevant, devoid of those implications it originally had with respect to the mystery of our own existence.

4.3. *The essence of life and OL scientific investigation*

OL research is energized by questions or visions that somehow touch the “big picture” of what life is, while remaining fully technical and scientific. One of these is, in our opinion, delivered by the notion of molecular self-association here discussed. Indeed, as the subtlety of the molecular basis of self-association is progressively discovered, we recognize new profound relationships between molecular properties and basic atomic physics, and thus between the OL and the basic structure of matter. Hence, the discovery of new powerful forms of molecular self-assembly that make life possible, would suggest that the inanimate, so often perceived far from our existence, carries in its basic structure a strong propensity to sprout in life. This in turn would give rise to a sense of purposefulness of the intimate structure of the Universe, a perception that is non-scientific by itself but that would be certainly stimulated by science.

Another very interesting question bridging scientific research to the essence of life, originally proposed by J. Monod in *Chance and necessity* [73], is how could purposeful living beings have emerged from a causal universe. The behavior of living beings, even simple ones such as bacteria, is much better described in terms of their purpose rather than by describing the causal pathways leading to their complex behavior. We could synthetically say that bacteria have strategies to attain their goals that are to survive and multiply even e.g., when their medium has been changed from glucose rich to glucose poor. Or we could describe the complex causal cascade of signaling leading to the expression of enzymes adequate to metabolize the new molecules they are lacking. Very clearly, the description in terms

of goals is a quite more efficient and synthetic description of the whole process. We can thus say that bacteria have goals. At a much higher level, we are certainly equipped by the sense of purpose and goal. How could it happen that this “teleonomic” capacity was raised by a causal, objective, purposeless inanimate world? We don’t want to discuss this issue here. We want to point to the fact that such a question brings within the horizon of the scientific research some of the ultimate expectations originally motivating the whole OL investigation. Indeed, this question has been continuously present in the field. Recently, A. Pross has proposed an intriguing answer [4,74]. According to him, when various entities are in dynamical competition (e.g. for food accrual), the dominating one behaves as if driven by the need of best efficiency in the competing element (gathering food), even though their essence remains causal, as everything within the natural world. An example of this concept (not proposed by Pross himself) is the formation of rivers through the erosion caused by the hydrogeological cycle. Rivers, and river basins “compete” to carry water downstream. The resulting arrangement of river paths is the one most efficient in optimizing the efflux of water, in turn incrementing erosion and thus stabilizing the basin capturing water. Could we synthetically say that the purpose of a river is to carry water downstream? Is this the way purpose entered the biological world? This is a challenging question stimulating thoughts far beyond the specifics of scientific research.

Is OL scientific research aiming to unveil the essence of life? Undeniably, such a goal is not within the methodological possibilities of science. However, at some level, this is indeed what scientists are after. There are visions underlying the OL research that have the power of affecting our concept of life even outside the scientific perspective, such as the notion that life could be found to be intimately related to the structure of matter through molecular self-association, or else such as the notion that the emergence of purposeful beings in a causal universe could be traced to simple competing kinetic processes. These visions are paradigmatic examples of how creativity works in science: new powerful concepts carry the notion that new important findings may be discovered, possibly bringing about far-reaching cultural implications. This generates expectation, the basis for a new flourishing of ideas.

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