Looking for the Primordial Genetic Honeycomb

Enzo Gallori · Elisa Biondi · Sergio Branciamore

Abstract All life forms on Earth share the same biological program based on the DNA/ RNA genomes and proteins. The genetic information, recorded in the nucleotide sequence of the DNA and RNA molecule, supplies the language of life which is transferred through the different generations, thus ensuring the perpetuation of genetic information on Earth. The presence of a genetic system is absolutely essential to life. Thus, the appearance in an ancestral era of a nucleic acid-like polymer able to undergo Darwinian evolution indicates the beginning of life on our planet. The building of primordial genetic molecules, whatever they were, required the presence of a protected environment, allowing the synthesis and concentration of precursors (nucleotides), their joining into larger molecules (polynucleo- tides), the protection of forming polymers against degradation (i.e. by cosmic and UV radiation), thus ensuring their persistence in a changing environment, and the expression of the "biological" potential of the molecule (its capacity to self-replicate and evolve). Determining how these steps occurred and how the primordial genetic molecules originated on Earth is a very difficult problem that still must be resolved. It has long been proposed that surface chemistry, i.e. on clay minerals, could have played a crucial role in the prebiotic formation of molecules basic to life. In the present work, we discuss results obtained in different fields that strengthen the hypothesis of a clay-surface-mediated origin of genetic material.

Keywords early genetic material · clay minerals · clay–nucleic acid complexes · surface-mediated origin of genetic material · prebiotic evolution

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Introduction

What is life? The question raised by Schrődinger (1944) more than 60 years ago still remains unanswered because of the difficulty that natural sciences have in defining a specific boundary between non-living and living matter. However, if we look at all life forms on Earth, we see that they share the same biological program, based on DNA/RNA genomes and proteins. The genetic information, recorded in the nucleotide sequences of DNA/RNA, supplies the language of life and by the steps of transcription and translation leads to the production of the amino acid sequence of a polypeptide. Moreover, the specific sequence of nucleotides in DNA is handed down, by the replication process, from one generation to the next, ensuring the perpetuation of the genetic information. These are the fundamental aspects of life, and although other features are necessary to life, the presence of genetic material is absolutely essential. The appearance in an ancestral era of a polymer able to replicate and evolve, from the Darwinian point of view, marks the beginning of life. Hence, the explanation of the formation of genetic material is fundamental for any discussion on the origin of life on our planet and perhaps any other.

The formation of a primordial genetic polymer, whatever it was, would have required specific conditions, including the synthesis and availability of precursors (nucleotides), the joining of these precursors into larger molecules (polynucleotides), and their protection against degradation (by cosmic and UV radiation), thus ensuring their persistence in a changing environment (Nisbet and Sleep 2001; Joyce 2002). The occurrence of these steps implies the presence of a protected niche where the genetic molecule could not only originate but also express its biological potential, i.e. the ability to self-replicate and evolve. Determining how these steps occurred and how the primordial genetic molecules originated on Earth is a very difficult problem that still must be resolved. Classical research in this field has focused on processes in aqueous solutions (Lazcano and Miller 1996; Delaye and Lazcano 2005), in the belief that building blocks of the biomolecules could be readily obtained by chemical reactions involving simple organic compounds present on the Earth's surface. However, the fact that the right components were present in primeval habitats is not sufficient by itself to explain the appearance of complex molecules. The creation of these macromolecules required the polymerization of single components. As biological polymers are generally formed by dehydration, it is difficult to conceive that complex macro-molecules could have originated by random collisions in the presence of a high concentration of water, as in these conditions hydrolysis is favoured, not polymerization (Pace 1991). This is particularly evident in the case of RNA molecule, which is supposed to have been the first genetic molecule to appear on Earth, in the era known as the "RNA World" (Gilbert 1986; Joyce 2002; Orgel 2004). The presence of 2′-OH group in the ribose renders the RNA chains particularly susceptible to hydrolysis (Doudna and Cech 2002).

It has long been proposed that surface chemistry on clays, or other minerals, was involved in the prebiotic chemical evolution that culminated in the origin of life. In 1951, J.D. Bernal suggested that clay minerals could have bound organic molecules from the surrounding water, concentrating them and protecting the molecules against destruction by high temperatures and strong radiation. In recent years, numerous observations have reinforced this hypothesis. Ertem and Ferris (1996) and Ferris (2002) demonstrated the polymerization of oligonucleotides up to the length of a small ribozyme on montmorillonite clay. Smith (1998) and Parsons et al. (1998) provided a theory for the assembly of organic molecules on the surface of silica-rich minerals resembling zeolites. In addition, experimental data in the field of molecular microbial ecology have strengthened the

hypothesis of a surface-mediated origin of life. Studies carried out on the "fate" of DNA in soil habitats have indicated that DNA originating from dead or living cells can persist for a long time in the environment, without losing its biological activity (i.e. the capacity to transform competent bacterial cells), as a result of its association with clay minerals (Gallori et al. 1994; Stotzky et al. 1996). These observations suggest that mineral surfaces could have played a crucial role in the formation and preservation of genetic material on the early Earth.

In the following sections, we report the results of our investigations on the association between different nucleic acids and clay minerals, and on the physical–chemical and biological characteristics of the resulting clay–nucleic acid complexes.

Clay–Nucleic Acid Complexes

Clay–nucleic acid complexes were prepared by reacting nucleic acids of different chemical composition and molecular structure (DNA, RNA) with two clay minerals, montmorillonite (M) and kaolinite (K), as previously described (Franchi et al. 1999; Franchi et al. 2003; Franchi and Gallori 2005). The resulting complexes were studied with regard to: (a) the equilibrium adsorption of nucleic acids on clay minerals; (b) the nature of the interaction between nucleic acids and clay; (c) the role played by cations in the adsorption process; (d) the environmental persistence in the presence of both biotic and abiotic degrading factors; (e) the ability of clay-adsorbed nucleic acid molecules to transmit their genetic information; (f) the ability of clay-adsorbed polymers to interact with surrounding molecules; and (g) the ability of a clay-adsorbed hammerhead ribozyme to perform a specific catalytic reaction ("self-cleavage" reaction).

The complexes were found to possess peculiar physical–chemical and biological characteristics.

Physical–chemical characteristics

Nucleic acids, differing in size and molecular structure, were rapidly adsorbed on M and K, with a maximum equilibrium adsorption after 90–100 min of contact. The tertiary structure and molecular weight of a nucleic acid molecule influenced both its adsorption and binding on clay (Franchi et al. 1999), while adsorption was not affected by the molecule's base composition (G+C content), or type of molecular end (blunt or cohesive) (Pietramellara et al. 2001). The adsorption was greater on M than on K. Extensive washing of the clay–nucleic acid complexes with H_2O partially (60–70%) removed the nucleic acid initially added, indicating that bonds with different strengths (strong and weaker) were formed between the nucleic acid molecules and clay minerals (Khanna and Stotzky 1992; Gallori et al. 1994). The Scatchard-plot analysis of the adsorption processes revealed two different types of binding sites between the nucleic acid molecule and clay surface, characterized by different affinities (Pietramellara et al. 2001). The results indicated that adsorption of different nucleic acids was strongly dependent on both the molecular characteristics of the nucleic acid and the chemical nature of the clay. To determine where nucleic acids are adsorbed on clay minerals and the nature of the interaction, we investigated the complexes by transmission and scanning electron microscopy (TEM, SEM), X-ray diffraction (X-RD), and Fourier transform infrared (FT-IR) spectroscopy (Franchi et al. 1999). TEM and SEM observations indicated that DNA strands were associated with several points of the edges of

clay particles, and the molecules appear to lie on the planar surface of the mineral particles. The lack of penetration of nucleic acid molecules into M and K was also suggested by X-RD. The analysis showed that none of the nucleic acid molecules significantly intercalated the Al–Si layers of the two clays, and that the adsorption occurred primarily on the external surface of the clay mineral. The FT-IR analysis of the complexes indicated that the conformation of the DNA and RNA changed when they were adsorbed on the clay minerals. In particular, DNA showed a transition from the B to A form, which could account, at least partially, for the resistance of clay–nucleic acid complexes to degradation in the environment. The adsorption/binding of nucleic acids on the mineral particles was promoted by the presence of cations (Franchi et al. 2003). The cation concentration required for adsorption depended on both the valence of the cations and the tertiary structure of the nucleic acid molecules. Double-stranded nucleic acid needed higher cation concentrations than single-stranded ones to establish the interaction with clay, and divalent cations were more efficient than monovalent ones in mediating this adsorption. Adsorption to the clay occurred only when both nucleic acids and cations were present, indicating that cations are directly involved in the formation of nucleic acid–clay complexes. The cations act as a "bridge" between the nucleic acid molecule and the clay particle neutralizing the negative charges present on both the phosphate group of the genetic polymer and the mineral surface.

Biological characteristics

Chromosomal and plasmid DNA bound on M and K were capable of transforming competent bacterial cells for a long period of time (Gallori et al. 1994; Stotzky et al. 1996). Thus, the adsorption/binding of DNA on clay minerals does not prevent their biological activity, but rather enhances the persistence of the genetic information in the environment, even in the presence of various degrading factors. DNA and RNA bound on M and K were protected, to some extent, from degradation by specific (DNase and RNase) and aspecific nucleases (restriction endonucleases) (Gallori et al. 1994; Franchi and Gallori 2005). The finding that the half-life of clay-adsorbed nucleic acid molecules (DNA, RNA) is higher than that of free molecules could solve, at least in part, one of the recurring problems with the evolution of genetic molecules in a primitive hostile environment characterized by various degrading factors (high temperature, hydrolysis, harsh radiation). Results of studies on the effect by UV and X-ray radiation support this observation. Free and clay adsorbed DNA were affected differently by UV and X-rays radiation. Clay-adsorbed DNA showed increased resistance to both UV photons and X-rays, while free DNA was severely damaged (Scappini et al. 2004; Ciaravella et al. 2004). These results could help understand to what extent critical conditions affected the evolution of prebiotic molecules toward complex systems. As there was no ozone layer in the primordial Earth (Margulis and Sagan 1986), it is necessary to hypothesize the existence of some kind of ground protection of the originating genetic information against the highly penetrating X and UV solar emissions. In this regard, the adsorption of primordial genetic polymers on ubiquitous clay minerals could have been a key step in the preservation of genetic material against X-rays and UV photodegradation.

    DNA and 16S RNA adsorbed on clay were still able to act as template for molecular replication by Polymerase Chain Reaction (PCR) (Vettori et al. 1996) and Reverse Transcription and amplification (RT–PCR) (Franchi and Gallori 2005). This indicates: (a) the possibility of transferring sequence information through different generations of

molecules, corroborating the hypothesis that clay–nucleic acid complexes could have acted as a "storage" of genetic information on early Earth (Franchi and Gallori 2004); and (b) the possibility of the passage from a single-strand molecule to a double-strand one in a prebiotic context, supporting the hypothesis that early pathways to life involved the formation of a RNA-like molecule prior to the formation of the first DNA (Joyce 2002). Moreover, clay-adsorbed RNA (Poly[A]) was able to interact with complementary polymers (Poly[U]) present in the surrounding medium and to establish a specific association with them, probably via the association of complementary helices (Franchi and Gallori 2005). Also in this case, the adsorption/binding of nucleic acid molecules on clay does not hinder their ability to interact with other molecules present in the environment, allowing the occurrence of further organized steps. The possibility that RNA molecules with catalytic activity (ribozymes) could perform their activities when complexed with clay minerals was assessed by studying the behaviour of the self-cleavage reaction (Doudna and Cech 2002) of a so-called "hammerhead" ribozyme in the presence of M. These ribozymes, present in certain circular RNA molecules named viroids, which are the smallest plant pathogens known (Flores et al. 2004), contain an RNA motif that can form a hammerhead secondary structure responsible for the self-cleavage reaction. Since viroids are able to undergo replication without DNA intermediates and without coding for proteins, they are considered possible relics of the RNA World (Diener 2001).

The hammerhead ribozyme present in the sequence of the Avocado Sun-Bloch viroid (ASBVd) (Flores et al. 2000), is particularly useful for these studies, as it is able to perform the self-cleavage reaction in three different ways: (1) via a single-hammerhead structure, where the ribozyme sequence folds in the well-known catalytic structure; (2) via double-hammerhead structure, where two hammerheads associate to form a more stable and active structure; and (3) via a partial double-hammerhead structure, where the 5′-fragment, formed by a previous event of self-cleavage, interacts with an entire ribozyme to form a structure similar to the double-hammerhead. ASBVd/M complexes subjected to the self-cleavage reaction could still self-cleave, albeit with reduced efficiency, demonstrating that the adsorption of the ribozyme on clay does not eliminates its catalytic activity. ASBVd tightly bound on clay was also able to establish a close association with the 5′-fragment present in the surrounding environment, and this association resulted in enhancement of the catalytic activity of the ribozyme. This indicates the possibility not only of a specific interaction between molecules adsorbed on clays and free molecules in the environment, but also of the modification of their activities. In this case, the association allows acquisition of the correct catalytic conformation of the clay-adsorbed ribozyme, suggesting that clays could have acted as "prebiotic environmental chaperones".

Conclusions

The results obtained thus far suggest that clay-rich sediments would have been a good habitat on the primordial Earth in which the first genetic molecules could originate and accumulate. We can hypothesize that an RNA-like World could have formed in pores of rocks such as feldspars or zeolites (Parsons et al. 1998), in an environment rich in phosphate and in contact with aqueous soups containing simple organic molecules (Figure 1). This kind of honeycomb would have allowed not only the building of genetic polymers but also their protection against environmental degradation (by harsh UV and X-rays radiation) for the time necessary to evolve toward an increasingly complex molecular

organization. In particular, these molecules must have been able to self-replicate and to interact with surrounding molecules, triggering the molecular evolution that led to the first living cells.

Of course, a thorough understanding of the surface-mediated origin of life will require much further research (Ertem 2004). Many uncertainties remain with regard to the true composition of the Earth' early crust (Schoonen et al. 2004), the nature of chemical reactions under prebiotic conditions (Delaye and Lazcano 2005), and how the self-replicating molecules could have led to the encapsulation of a complex system of biochemical reactions in a membrane-like structure to form the first unicellular organism. Recent observations that clay particles can favour the spontaneous conversion of fatty acid micelles into vesicles, and the encapsulation in the latter of clay-adsorbed RNA molecules (Hanczyc et al. 2003; Hanczyc and Szostak 2004), seem to suggest a possible solution to this crucial problem.
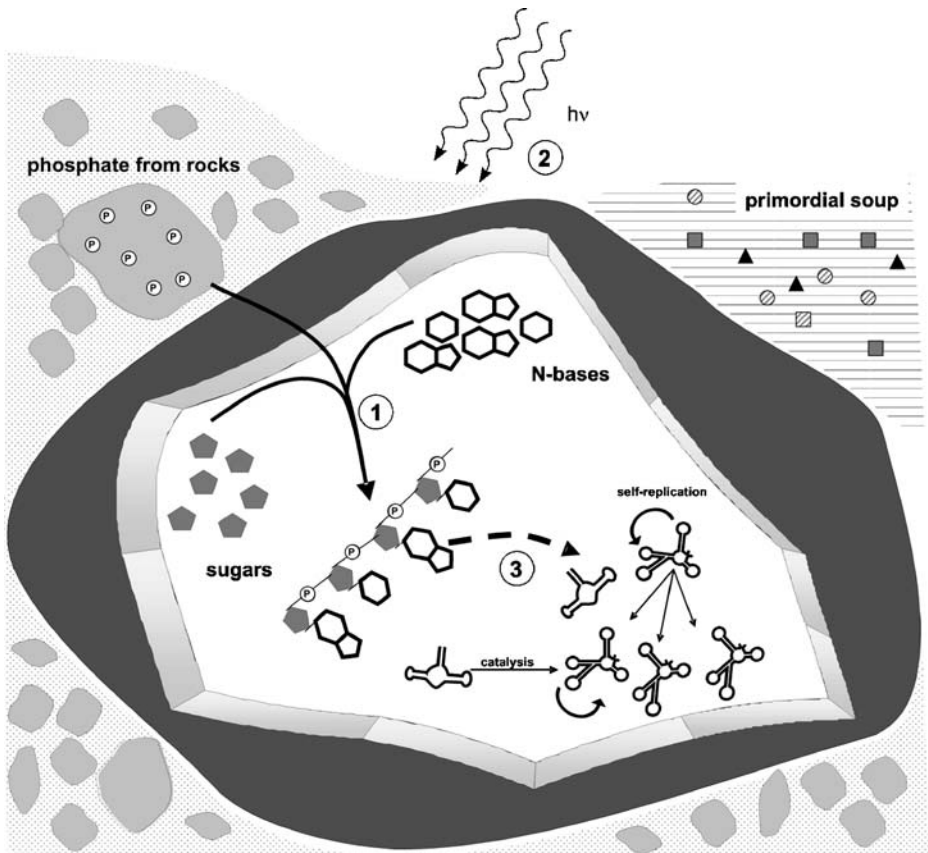

Figure 1 Schematic representation of possible reactions leading to the primordial genetic material in an ancestral clay-rich habitat:(*1*) building of genetic molecules; (*2*) protection against degradation (by UV and X-ray radiation); (*3*) evolution toward increasing molecular organization (self-replication and catalysis).

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