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Review

Formamide and the origin of life

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Abstract

The complexity of life boils down to the definition: "self-sustained chemical system capable of undergoing Darwinian evolution" (Joyce, 1994) [1]. The term "self-sustained" implies a set of chemical reactions capable of harnessing energy from the environment, using it to carry out programmed *anabolic* and *catabolic* functions. We briefly present our opinion on the general validity of this definition.

Running anabolic and catabolic functions entails complex chemical information whose stability, reproducibility and evolution constitute the core of what is dubbed *genetics*.

Life as-we-know-it is made of the intimate interaction of metabolism and genetics, both built around the chemistry of the most common elements of the Universe (hydrogen, oxygen, nitrogen, carbon). Other elements like phosphorus and sulphur play important but ancillary and potentially replaceable roles.

The reproducible interaction of metabolic and genetic cycles results in the hypercycles of organization and de-organization of chemical information that we consider living entities. In order to approach the problem of the origin of life it is therefore reasonable to start from the assumption that both metabolism and genetics had a common origin, shared a common chemical frame, were embedded in physical–chemical conditions favourable for the onset of both.

The most abundant three-atoms organic compound in interstellar environment is hydrogen cyanide HCN, the most abundant three-atoms inorganic compound is water H_2O. The combination of the two results in the formation of formamide H_2NCOH. We have explored the chemistry of formamide in conditions compatible with the synthesis and the stability of compounds of potential pre-genetic and pre-metabolic interest. We discuss evidence showing (i) that all the compounds necessary for the build-up of nucleic acids are easily obtained abiotically, (ii) that essentially all the steps leading to the spontaneous generation of RNA are abiotically possible, (iii) that the key compounds of extant metabolic cycles are obtained in the same chemical frame, often in the same test tube.

How close are these observations to a plausible scenario for the origin of life?

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1. Introduction

1.1. The need for a unitary physical–chemical frame

It is common belief that we will never know exactly how life originated, but that we will be able to reconstruct the plausible steps and the physical–chemical rules on which the first reactions were based and the kick-starting compounds formed.

The onset of the complex "self-sustained chemical system capable of undergoing Darwinian evolution" [1] that we dub life was presumably favoured by the cooperative encounter of necessarily robust physical–chemical processes. The early [2] appearance of living entities, the conservativeness of their basic principles, and their biological universality support this view.

The approach to the understanding of the first pre-biological scenarios is variegate. Multidisciplinarity, usually fertile, caused in this case the field to be split in several doctrinarian attitudes simplified by the aphorisms "genetics-first" [3–5], "metabolism-first" [6–9] or, even, "membranes-first" [10]. An entry in the literature of this debate is in [11]. In recent years the opposition between the two approaches has been overstepped by more unitary experimental and theoretical frames, taking into account energetic, evolutionary, proto-metabolic and ur-environmental aspects [12–22]. The productive interaction between vesicles and RNA replication was reported [23]. Nevertheless, a unitary and simple chemical frame is needed that would afford in a single "warm little pond" [24] both the precursors of the synthetic pathways eventually leading to RNA [25] and the key components of the central metabolic cycles, possibly connected with the synthesis of fatty acids [26]. The singleness of such prebiotically productive chemical process would partake of Darwinian advantages over more complex fragmentary chemical systems.

By referring to Darwin's suggestive image, we do not intend to embrace any specific prebiotic scenario, be it a drying lagoon, an intertidal beach or hydrothermal system activated by meteorite impact. The fact that the majority of the reactions that we describe are favoured by temperature $> 100\,^{\circ}C$ (typically $160\,^{\circ}C$), by high pH and by the presence of minerals rather confers to the Darwinian image (which, given the times, was necessarily vague) the attributes of milder hydrothermal vents. Definition of a precise prebiotic scenario is beyond our present scope.

1.2. (Pre)genetics and (pre)metabolism

Focusing on the one-carbon atom formamide H_2NCHO (thus well within the frame of reference of the chemistry of hydrogen cyanide HCN), we discuss studies showing the plausibility of each single step along the route leading from the synthesis of nucleic bases to the formation of long RNA chains in the simplest possible prebiotic perspective.

We describe studies on the synthesis from formamide of nucleic bases and of acyclonucleosides; on the phosphorylation of nucleosides in formamide, including the formation of cyclic nucleotides; on the roles exerted by numerous classes of mineral catalysts on these reactions. We detail the non-enzymatic polymerization in water of cyclic purine nucleotides yielding RNA oligomers.

First, we report that all the necessary nucleic bases are afforded by formamide chemistry [27]. Then, we comment on the fact that the origin of nucleosides is the weakest link in this possible chain of events. Recent findings suggest a solution [28], for conditions probably different from those that supposedly characterized prebiotic processes (namely: simplicity, robustness and availability of abundant precursors). Formamide chemistry allows the formation of acyclonucleosides in non-demanding conditions [29]. As for the next step toward (pre)genetic complexity, formamide chemistry also carries on phosphorylation of nucleosides [30].

Passing to water chemistry, we describe previous studies and recent advancements on the oligomerization of cyclic purine nucleotides, both in the absence and in the presence of complementary template sequences, and on the non-enzymatic joining of oligomers to form long RNA chains [31]. Interestingly, we have recently observed that RNA polymerization may also occur in the presence of formamide (see Section 2.5).

These processes do not imply that one can induce the abiotic polymerization of RNA in a single test-tube in a single set of conditions, here and now, starting from one-carbon simple molecules. However, the plausibility of each

necessary step is described in the frame of one single type of chemistry in relatively mild physical–chemical conditions and in a time scale compatible with the accumulation of (pre)genetic chemical information.

1.3. Genetic cycles or metabolic cycles?

Which came first? The condensation reactions within the range of the thermodynamic driving force provided by the free energy of HCN as starting material open a convergent route to the non-enzymatic syntheses of nucleic bases and of major constituents of key metabolic cycles. We will describe studies on the synthesis of a series of connected carboxylic acids afforded by the same reactions in the same conditions with the same catalysts.

H_2NCOH formamide provides a robust and reactive source potentially feeding both chapters of the book of origins. Cooperation between cycles within a unitary chemical frame is thus made potentially possible.

2. Formamide

Formamide is the simplest naturally occurring amide. This compound contains in its structure all the elements, hydrogen, carbon, oxygen and nitrogen which, with the only exception of phosphorus and sulphur, are required for the synthesis of biomolecules [32]. As extensively reported in recent years, NH_2CHO is a ubiquitous molecule in the Universe. It has been detected in comets [33], as in the case of comet C/1995 O1 (Hale-Bopp) [34] and comet C/1996 B2 (Hyakutake) [35], in the solid phase on grains around the young stellar object W33A [36], in the galactic center sources Sagittarius SgrA and SgrB2 [37,38] and, in general, in the interstellar medium [39]. In addition, recent data suggest the presence of formamide on some celestial bodies in the solar system, including Titan and the Jupiter's satellite Europa, where a stratosphere of liquid formamide (pure or partially mixed with water) below the frozen surface of the mantle has been hypothesized [40–42].

Several theoretical and experimental studies have been performed to interpret the formation of formamide in space conditions [43,44]. As an example, the formation of formamide and of other organics has been reported starting from gas mixtures of methane and nitrogen by proton irradiation (PI) [45], from ice-mixtures of hydrogen cyanide (HCN), water (H_2O) and ammonia (NH_3) under ultraviolet irradiation (UV) [46], from mixtures of carbon monoxide (CO), NH_3 and H_2O during pyrolysis [47], and by photolysis of ices [48] (Fig. 1). Similarly, NH_2CHO can be synthesized in terrestrial conditions from mixtures of low molecular weight compounds, such as NH_3, formic acid (HCOOH), formic ester derivatives (HCOOR), CO and alcohols, under both catalyzed and uncatalyzed experimental conditions [49].

In particular, heterocycles containing the imidazole ring, like thiamine hydrochloride [50], ammonium methoxide [51], sodium methoxide [52], glycerine [53], different Lewis and Bronsted acids and bases [54], have all been used as catalysts for the synthesis of NH_2CHO in different experimental conditions. Among these syntheses, the one that has probably greatest significance in terms of prebiotic chemistry, is the formation of NH_2CHO by hydrolysis of HCN (Fig. 1).

HCN is one of the most studied chemical precursors of biomolecules [55]. After the adsorption of HCN in water, two processes may occur: the polymerization to biomolecules or poly(hydrogen cyanide) derivatives and the hydrolysis to NH_2CHO. The hydrolysis predominates in dilute solutions, while polymerization takes over at higher concentrations [56]. The steady state concentration of HCN in the primitive ocean was found to be too low for polymerization, thus favouring hydrolysis to NH_2CHO [56,57]. Similarly, NH_2CHO can be hydrolyzed to ammonium formate ($NH_4^+HCOO^-$) at a meaningful rate. However, one has to take into account the fact that formamide can be regenerated by other synthetic routes described above and that, at difference from HCN, it can be easily concentrated by evaporation of water, having a boiling point of 210 °C and very limited azeotropic effects [58].

Another interesting property that characterizes the NH_2CHO as prebiotic precursor is related to the possibility of a partial degradation under the reaction conditions to generate a panel of low molecular weight compounds which are useful intermediates in the prebiotic synthesis of biomolecules, thus increasing the network of possible transformations [58]. This property points to NH_2CHO as a multifunctional prebiotic precursor.

As an example, NH_2CHO is thermally decomposed either to NH_3 and CO or to HCN and H_2O. The formation of HCN is usually favoured in the presence of suitable catalysts, while in their absence the reaction forming NH_3 and CO predominates [59]. Further decomposition products are also detected. These include poly(hydrogen cyanide) derivatives (PHC) potentially producing nucleic bases under hydrolytic conditions [60]. Moreover, in conditions of radical

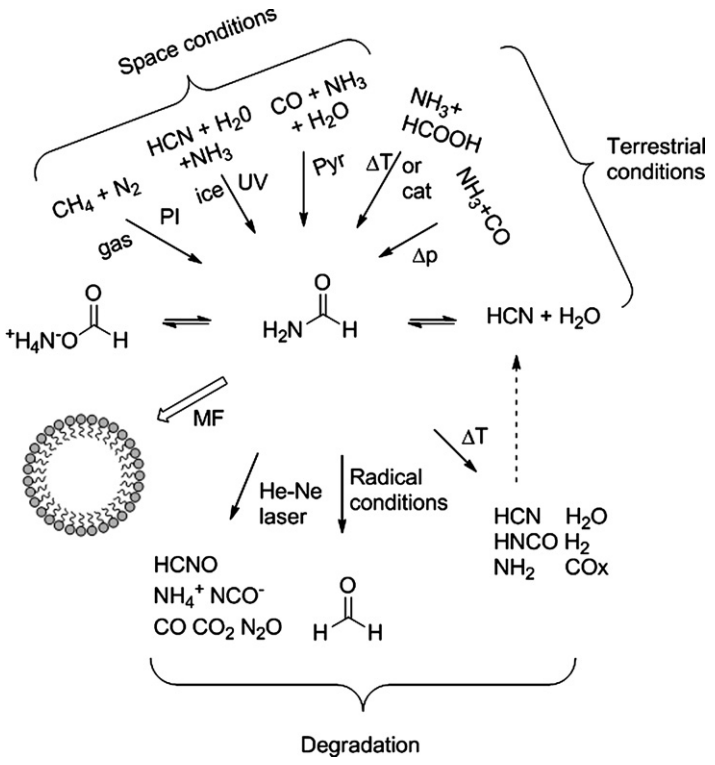


Fig. 1. The basic prebiotic chemistry of formamide. PI: Photon irradiation. UV: Ultraviolet irradiation. Pyr: Pyrolysis. ΔT and ΔP: High temperature and/or high pressure. Cat: Reaction performed in the presence of catalysts. MF: Micelle formation.

reactions catalyzed by titanium dioxide (TiO_2), NH_2CHO can be degraded to formaldehyde H_2CO, a compound which is currently the most studied prebiotic precursor for the origin of sugars.

The synthetic potential of formamide is also increased by specific physical conditions, such as by microwave radiation [61]. Due to its high dielectric constant, formamide is also a good solvent for polar organic and inorganic compounds with potential catalytic activity in prebiotic processes [62].

Finally, NH_2CHO (and others NH_2CHO derivatives, such as *N*-methyl formamide and *N*,*N*-dimethyl formamide) can play an important role in the phenomenon of compartmentalization of the first cell considerably increasing micelle formation of ionic surfactants (sodium caprylate, sodium laurate, sodium palmitate and sodium stereate), the driving force for the supra-molecular organization being correlated with the solvophobicity of the system [63].

2.1. Syntheses of nucleic bases from formamide

As mentioned above, H_2NCHO can be decomposed to a large panel of low molecular weight compounds (carbon oxides, ammonia, isocyanate, and others) under various experimental conditions. All these compounds are possible intermediates in the prebiotic synthesis of nucleic acids, thus increasing the network of possible transformations [64,65]. The chemical potential of H_2NCHO in prebiotic chemistry was, for a long period of time, considered to be limited to the synthesis of a small number of heterocycles including, as a nucleic base, only adenine [66,67]. Seminal studies have shown that formamide oligomers, ammonia, and HCN were involved in the mechanism of formation of adenine by a multi-steps condensation process [68,69].

A meaningful improvement in this chemistry was obtained in 2001 through the analysis of the catalytic effect of simple metal oxides in a thermal condensation process. Minerals and metals oxides were largely diffused on the surface of the primitive Earth and probably played a key role in the synthesis, selection, concentration and organization of simple organic compounds to complex biomolecules [70].

The gentle warming of H_2NCHO at 160 °C in the presence of catalytic amounts of calcium carbonate ($CaCO_3$), alumina (Al_2O_3), silica (SiO_2), and zeolite (Y type) yielded cytosine and *4(3H)*-pyrimidinone, in addition to purine

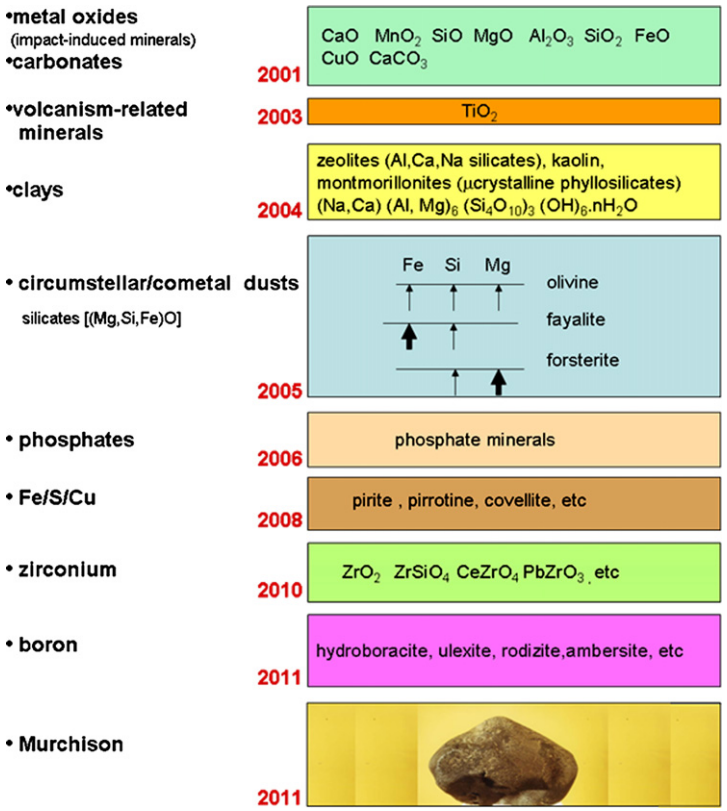


Fig. 2. Chronological evolution in the prebiotic chemistry of formamide from 2001 to 2011. The minerals and metal oxides used as catalysts in the condensation processes are reported. The arrows in the circumstellar/cometal dusts panel is a qualitative representation of the content of iron, silica and magnesium in the catalyst.

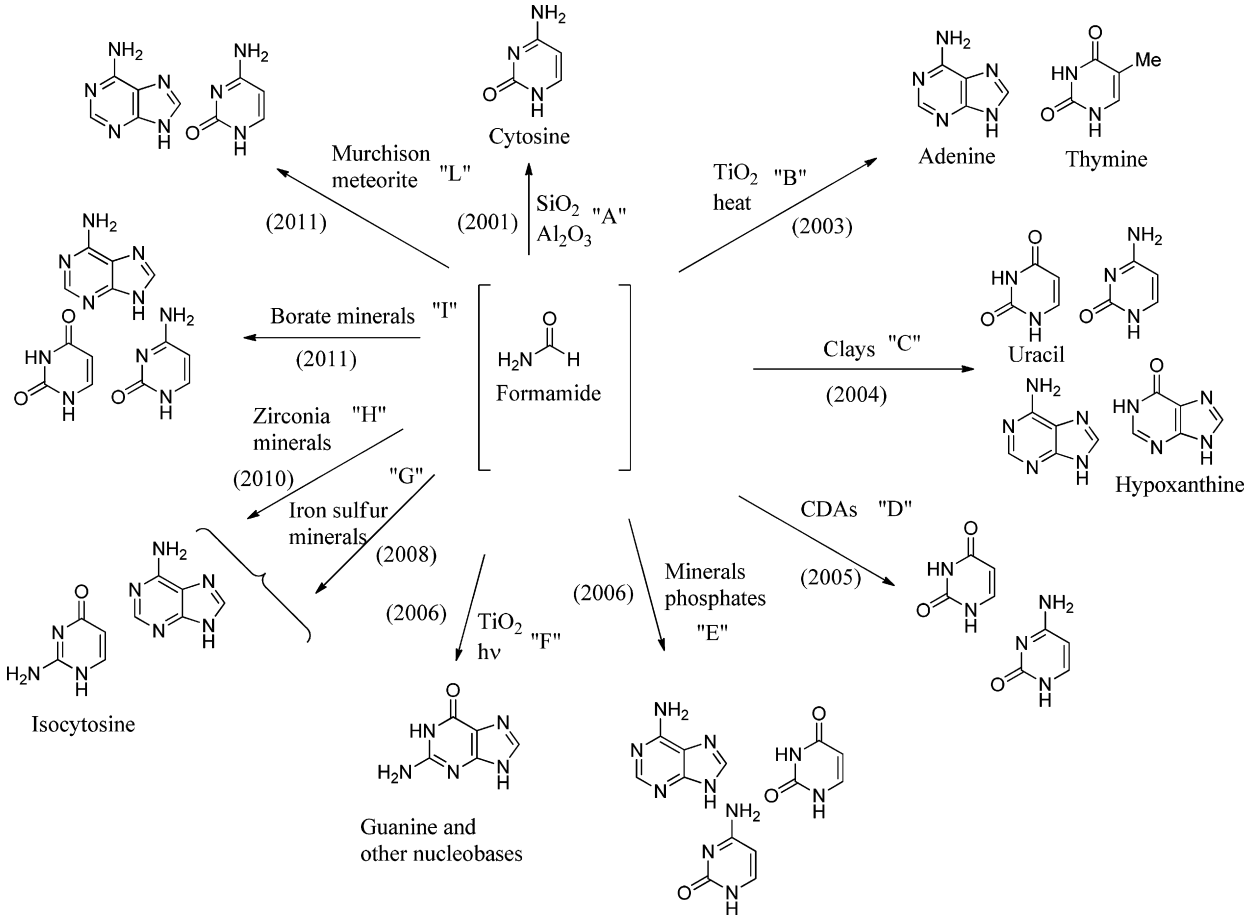
and adenine (Fig. 2 and Scheme 1 pathway "A") [71]. These data were the first example of the one-pot synthesis of cytosine starting from a one-carbon atom precursor as simple as H_2NCHO [72].

In 2003 thymine was synthesized from H_2NCHO in the presence of titanium dioxide (TiO_2) [29]. In this case three novel purine acyclonucleosides, in which the heterocyclic base is bonded to a sugar-like side-chain in the *N(9)*-position, were obtained in addition to 5-hydroxymethyl uracil (5-HMU). The presence of 5-HMU in the reaction mixture suggested a synthetic pathway for thymine, requiring the electrophilic addition of formaldehyde on the *C(5)*-position of uracil as intermediate, followed by a reduction step mediated by formic acid. In agreement with this hypothesis, traces of formaldehyde, formic acid and uracil were detected by gas chromatography-mass spectrometry analysis (Fig. 2 and Scheme 1 pathway "B") [73].

Uracil, adenine, cytosine and hypoxanthine were obtained in 2004 by thermal condensation of H_2NCHO in the presence of clays of the montmorillonite family (Fig. 2 and Scheme 1 pathway "C") [74]. Uracil was probably produced by deamination of cytosine through a catalysis due to the known acidic character of montmorillonites [75].

The ribonucleoside corresponding to hypoxanthine (inosine) is the starting material for the biosynthesis of adenosine and guanosine. 5-Aminoimidazole-4-carboxamide (AICA) and 5-formamidoimidazole-4-carboxamide (fAICA), the imidazole intermediates for hypoxanthine in the cell, were also detected in the condensation mixture [32,74]. Thus, the H_2NCHO/montmorillonite syntheses appeared to be the first documented example of a chemiomimetic system [76,77].

In 2005 the pyrimidine nucleobases uracil and cytosine were selectively synthesized from H_2NCHO and cosmic dust analogues (CDAs) of terrestrial olivines (from fayalite to forsterite). Purine derivatives were not detected under these experimental conditions. In particular, the efficacy and selectivity of the reaction was determined by the content of iron in the catalyst, suggesting a redox process as a key step in the formation of the pyrimidin-(*1H,3H*)dione scaffold (Fig. 2 and Scheme 1 pathway "D") [78].



Scheme 1. Synthesis of nucleobases from formamide, metal oxides and minerals: an overview. The compounds are indicated by their common name only when obtained for the first time, with the exception of adenine (see text).

In 2006, adenine, cytosine and uracil were obtained by warming H_2NCHO in the presence of one out of a large panel of mineral phosphates, confirming the possibility of the contemporaneous synthesis of purine and pyrimidine nucleobases under simple conditions (Fig. 2 and Scheme 1 pathway "E") [79,80].

At that time, only one of the five natural nucleobases was lacking from the panel of products obtained from H_2NCHO: guanine. This bias was partially solved in 2006, when experiments on UV light irradiation of H_2NCHO on the surface of a TiO_2 (001) single crystal at low temperature in ultra-high vacuum conditions showed (even though only tentatively) the formation of all five nucleic bases, including guanine (Fig. 2 and Scheme 1 pathway "F") [81]. The possibility to synthesize guanine from H_2NCHO was confirmed in 2010 by a combined UV-irradiation/thermal condensation process in the presence of phosphate minerals [82].

Minerals characterized by redox properties have been suggested as key effectors in the origin of the metabolic apparatus, as exemplified by the iron sulfides used in Wächtershäuser's model [83].

In this system FeS minerals had no catalytic function, being oxidized from FeS to FeS_2. The catalytic function of FeS minerals, with transfer of electrons from H_2 to CO_2 via FeS cluster as ferredoxin mimetics, was reported and analyzed in detail [84–86].

These minerals are able to catalyze the thermal condensation of H_2NCHO to yield nucleic bases. In 2008 the synthesis of adenine and isocytosine from H_2NCHO in the presence of different iron sulphur and iron–copper sulphur minerals (including pyrite and pyrrothite) was reported (Fig. 2 and Scheme 1 pathway "G") [27,87]. Isocytosine is a structural isomer of cytosine, recognizing both guanine (iCG) and cytosine (iCC) in reversed and normal Watson–Crick interactions, respectively [88].

A similar reaction pathway was observed in 2010 during the thermal treatment of H_2NCHO with zirconium minerals at 160 °C [89]. Under these experimental conditions, adenine and isocytosine were obtained (Fig. 2 and Scheme 1 pathway "H") in addition to urea, carbodiimide and various carboxylic acids, three of which (succinic, maleic and fumaric acids) are intermediates of the reductive version of the citric acid cycle (rTCA) (see Section 3). This cycle of autocatalytic processes is probably the most ancient anabolic core in intermediary metabolism [90,91]. Carbodiimide, a product of urea dehydration, is a well-known condensing agent able to catalyze the formation of the peptide and phosphoester bonds in proteins and oligonucleotides, respectively [92].

A large panel of nucleic bases (adenine, uracil and cytosine), including isocytosine, were synthesized in 2011 from formamide and borate minerals (Fig. 2 and Scheme 1 pathway "I") [93]. Borate minerals selectively favor the formation of ribose during the formose-like condensation process of formaldehyde and glycolaldehyde, due to coordination of the cis-diol moiety [94]. The formose reaction requires alkaline conditions, which is entirely compatible with an alkaline hydrothermal vents scenario. As a general trend, adenine was synthesized with anhydrous borates, while cytosine was synthesized with hydrates of borates, anhydrous borates, borocarbonate and borosilicates. For a classification of borate minerals, see [95]. As observed with zirconiun minerals, borate minerals catalyze the formation of four carboxylic acids including pyruvic acid, which is also an intermediate of the reverse citric acid cycle.

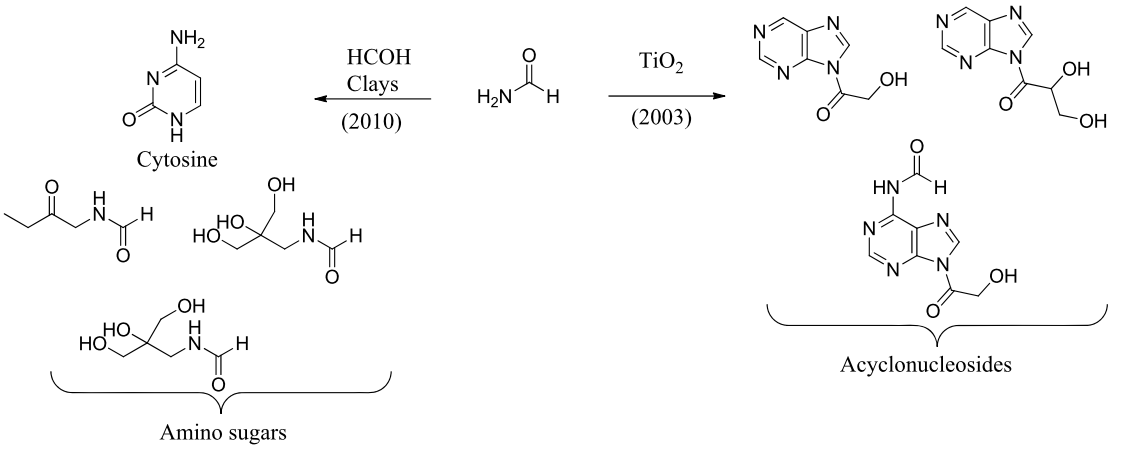
As a possible extension of this experimental model to prebiotic chemistry in space-wise conditions, uracil, adenine and isocytosine were synthesized in 2011 by heat-driven condensation of H_2NCHO in the presence of Murchison material. These data are of particular interest providing the first example on the catalytic effect of a meteorite on the synthesis of nucleic bases (Fig. 2 and Scheme 1 pathway "L") [96].

2.2. Plausible syntheses of nucleosides in prebiotic conditions

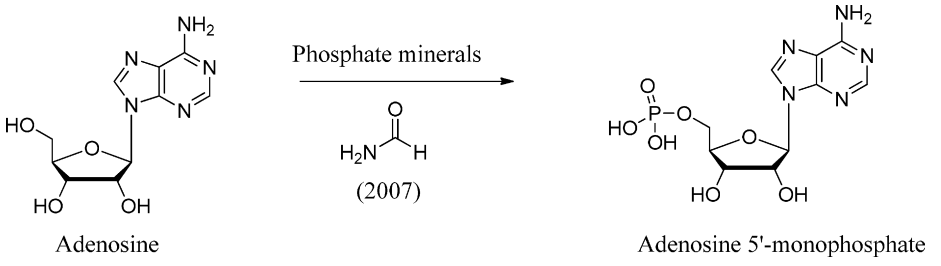
The next step towards the synthesis of nucleic acids requires the formation of nucleosides, compounds in which the nucleic base is linked to a sugar, ribose or 2′-deoxyribose for RNA or DNA, respectively. In principle, the synthesis of nucleosides can be achieved by three different reaction pathways: a) the direct formation of the glycosidic bond between a pre-formed nucleic base and a sugar; b) the construction of the nucleic base heterocyclic scaffold on a pre-formed sugar; c) the construction of the sugar on a pre-formed nucleic base. On the basis of existing data, the direct condensation of sugar and natural nucleic bases does not work efficiently under prebiotic conditions either for kinetic and thermodynamic reasons [97]. Examples of the two other reaction pathways of possible prebiotic interest have been reported: 1) β-ribocytidine-2′,3′-cyclic phosphate was synthesized with high stereoselectivity by a multistep process starting from glycoladehyde and cyanamide through a pentose amino-oxazoline derivative on which the cytosine scaffold was gradually built [28]. The use of amino pentose-oxazolines as intermediates in the synthesis of nucleoside derivatives is a well-known practice in medicinal chemistry [98]. These procedures generally require lengthy optimization of experimental conditions and efficient purification techniques of the various intermediates [98]; 2) different purine acyclonucleosides have been synthesized from H_2NCHO and TiO_2 by a one-pot domino process [29] including the *N*-formylation of the nucleobase at the *N*(9)-position followed by a formose condensation. In this process, the sugar side-chain grew adding single carbon units of formaldehyde. These data were further confirmed analyzing the thermal condensation of H_2NCHO in the presence of added formaldehyde and montmorillonites. In this latter instance a mixture of nucleic bases, sugars and amino sugars was obtained [26]. Amino sugars are useful intermediates for the construction of the nucleic base that can be obtained by incorporating the amino group through condensation reactions with one or more reagents, such as β-diketoesters (see Scheme 2).

2.3. Nucleoside phosphorylation

In the presence of a phosphate source at high temperature in formamide nucleosides are phosphorylated [30]. The phosphate source may consist of KH_2PO_4 or of one of several phosphate minerals. The most efficient phosphorylation was observed with KH_2PO_4 and hydroxylapatite, followed by Cu-containing phosphate minerals; namely, in the order, libethenite $Cu_2^{2+}(PO_4)(OH)$, cornetite $Cu_3^{2+}PO_4(OH)_3$, ludjibaite $Cu_5^{2+}(PO_4)_2(OH)_4$, reichenbachite $Cu_5^{2+}(PO_4)_2(OH)_4$. The optimal temperature observed was 90 °C, temperatures lower than 60 °C being inefficient, higher ones causing faster degradation of the compounds formed. Phosphorylation could occur at any of the three possible positions of the sugar: 2′, 3′ or 5′ although, in the case of adenosine, phosphorylation occurred mainly in the 5′-*O* ribose position with formation of adenosine-5′-monophosphate.



Scheme 2. One-pot synthesis of acyclonucleosides and amino sugars from formamide.



Scheme 3. Nucleoside phosphorylation in formamide in the presence of phosphate minerals.

Interestingly, also 2′,3′ and 3′,5′ cyclic forms were observed. Upon incubation for several hundred hours at 90 °C the cyclic forms were the majority of the intact phosphorylated nucleotides remaining, due to their higher stability relative to the open forms in the phosphorylation conditions. It has not escaped our attention the possibility that these non-enzymatic phosphorylation reactions could provide a means to form and preferentially accumulate, in the form of cyclic nucleotides, a source of naturally activated precursors for abiotic polymerization (see Scheme 3).

2.4. *Abiotic RNA*

Different solutions for the origin of abiotically obtained RNA polymers have been proposed and reviewed [99–101] and conditions for the promotion of their polymerization have been defined [102,103]. The actual prebiotic relevance of several of these solutions was criticized [97], due to their requirement for highly activated precursors whose formation entails complex chemistry.

The lipid-assisted synthesis of RNA-like polymers from mononucleotides [104], does not ask for a pre-activation step and is bereft of this limitation. For an in-depth analysis of this topic see [105].

The difficulty in understanding and recreating the spontaneous generation of RNA in a simple-chemistry frame and in the absence of enzymatic activities has stimulated the proposal of polymers with totally different structures, yet maintaining their nucleic bases-based specificities. Peptide Nucleic Acid (PNA) [106] stands out (besides its *per se* interest) for its plausibility. Data on its prebiotic valence have been gathered [106]. A different approach consists of the exploration of enhancers and effectors of ribozymic activities. Very interestingly, nucleic bases-based activities have been reported, both in normal and extreme pressure conditions [107].

Cyclic phosphate bonds were explored previously as a potential source of RNA polymers and of formation of phosphodiester bonds for the connection of preformed oligonucleotides. In particular, 2′,3′ cyclic phosphate bridges were reported to promote the formation of phosphodiester bonds connecting preformed oligonucleotides bound to the appropriate sequence-complementary strands in the absence of catalysts [108]. In this system, in addition to the capacity to form phosphate bridges, the higher stability of 3′,5′ bonds relative to 2′,3′ bonds in double-stranded RNA

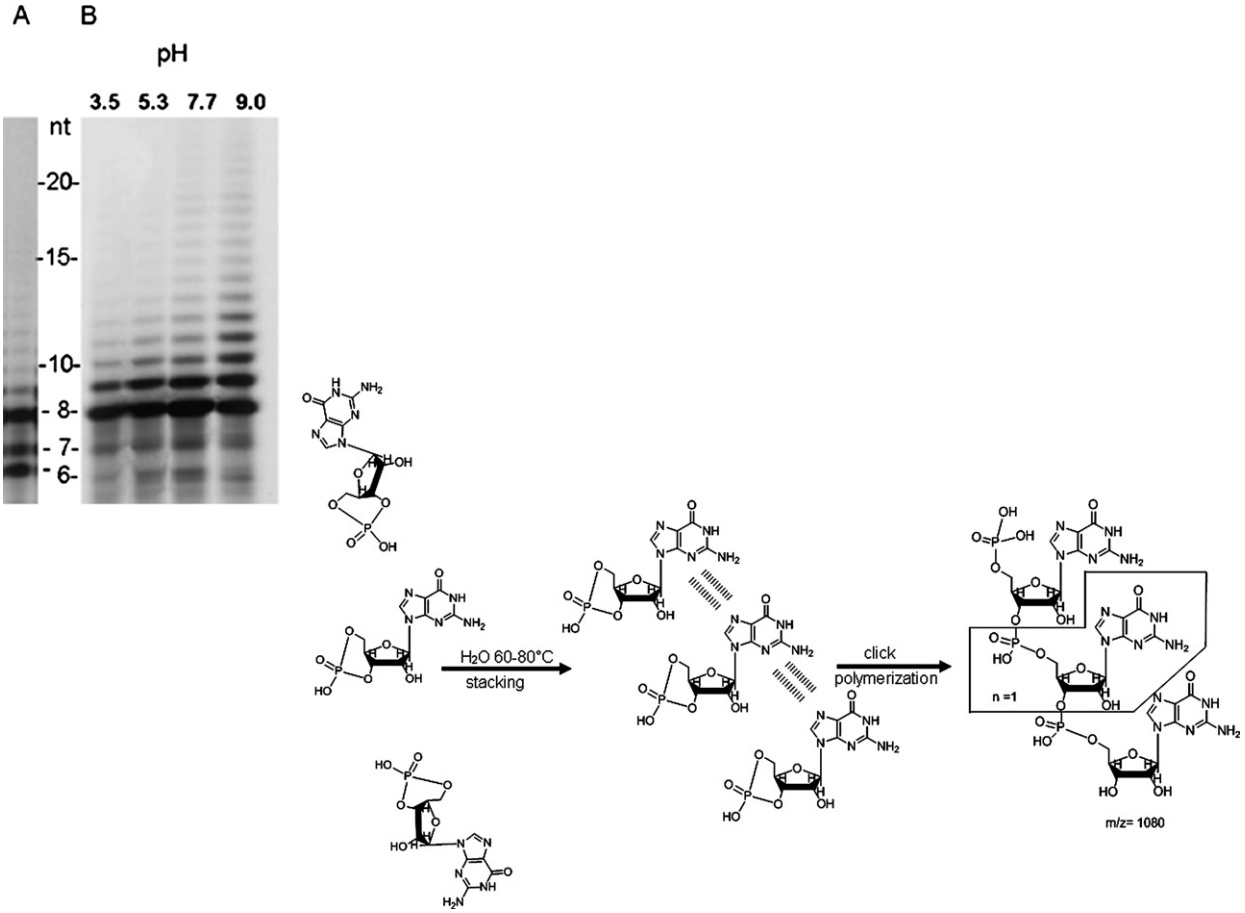


Fig. 3. Non-enzymatic polymerization of 3′,5′ cGMP in water. Panel A shows the population of different length oligomers obtained by dissolving 3′,5′ cGMP in water pH 6.0 at 4 °C and precipitated, as described [31], after a handling time of 5 seconds. Numbering refers to the length in nucleotides, determined by comparison with a ladder produced by hydrolysis of a G24 oligomer (not shown, as detailed in Ref. [31]). Panel B: the products of polymerization allowed to proceed for 10 min at 85 °C at the indicated pH. The different pH values were obtained by the use of the appropriate 10 mM Tris-HCl buffer. Panel C: free floating 3′,5′ cGMP monomers (left) have strong tendency to form stacked structures (center). Part of these undergo ring-opening and bond formation with the adjacent unit at 5′. The resulting molecules are of the type 5′ pG[pG]$_n$pG3′, where $n = 1$ is the trimer, $n = 2$ is the tetramer, and on. See Section 2.5.

structures was observed [109]. In the presence of 1–2 diamino ethane at alkaline pH the self-polymerization of 2′,3′ cyclic adenosine mono-phosphate (2′,5′ cAMP) was described [110]. The non-enzymatic ligation of short-chained 2′–5′- or 3′–5′-linked oligoribonucleotides on 2′–5′- or 3′–5′-linked complementary templates was also reported [111]. In spite of this observed capacity of both 2′,3′ and 3′,5′ cyclic phosphate bonds to promote covalent linkage between RNA segments, the polymerization of 3′,5′ cyclic nucleotides was to our knowledge not reported.

We observed [31] that 3′,5′ cyclic guanosine mono-phosphate (3′,5′ cGMP) and to some extent also 3′,5′ cAMP, afford short oligomers upon solubilization in water. Upon treatment in various conditions (see below), these oligomers undergo further polymerization. Here we describe the key features of this reaction focusing on the polymerization of 3′,5′ cGMP, the more efficient of the two.

2.5. *The 3′,5′ cGMP polymerization reaction*

3′,5′ cGMP polymerizes spontaneously by base-catalyzed trans-phosphorylation of stacked monomers (Fig. 3). We describe below the whereabouts of this reaction [31].

The simplest possible polymerization protocol. Addition of pure water or of Tris-buffered water to 3′,5′ cGMP resulted in the rapid formation of oligomers (Fig. 3, panels A & B). 3′,5′ cGMP was obtained from providers (Sigma

Aldrich and Carbosynth) both in the H^+ and Na^+ forms. The efficiency of the polymerization observed with the Na^+ form was at least one order of magnitude lower than that observed with the H^+ form, both in terms of the amount of oligomer formed and of its N_{avg} length. The sample was treated as detailed in [31], the procedure consisting in short of alcohol precipitation, terminal labeling by T4 polynucleotide kinase, phenol extractions and denaturing gel electrophoresis.

An initial reaction is observed that is faster than the 5 seconds necessary for the handling of the sample. This poses the question of the possible presence of preformed oligomers in the starting 3′,5′ cGMP material. A rigorous verification of this possibility is difficult. The starting material was analyzed by Phosphorus Nuclear Magnetic Resonance analysis (^{31}P NMR) and by HPLC. With both techniques, traces of polymerized material were observed. Taking into account that even if no trace of pre-existing polymer were observed, selective precipitation phenomena of eventual traces of pre-existing polymerized material could not be totally excluded, we conservatively consider from these analyses that the starting material is not completely pure.

Independently of the unverifiable presence of trace amounts of preformed polymers in the starting material, an active polymerization reaction takes place upon solubilization of the cyclic monophosphate in water. This polymerization reaction is non-fastidious and it is easily reproduced, provided that one takes into account that the Na^+ form of cyclic nucleotides polymerizes very poorly. The reason of the low reactivity of the Na^+ form is presumably the strong interaction of the nucleotide with Na^+ held in place by four coordinated molecules of water [112]. Due to the low yield of the reaction, sensitive analytical methods should be applied. Several parameters characterizing the polymerization of 3′,5′ cGMP in water were determined, as follows:

Temperature: The optimal temperature for the formation of G oligomers is 80 °C, higher temperature probably interfering with an initial step of stacking of the cyclic monomers [113]. *pH*: alkaline pH favours the reaction. The optimal value determined is 9.0. *Stability*: in pure water at 80 °C polymerization steady state is rapidly reached, due to RNA instability at high temperature. Stability of RNA and of its precursors in water as a function of temperature was analyzed in detail [114]. *Concentration of the reacting species*: the optimal concentration of precursors was determined to be, at 80 °C, 6 mM. At concentrations higher than 10 mM precipitation phenomena hamper polymerization. The problem of the concentration of precursors in the generation of nucleic acid polymers is a major chapter of prebiotic chemistry. Its possible solutions depend largely on the scenario in which polymerizations are considered. A possible solution of general applicability is provided by the potent concentrating mechanism of both short and long nucleic acid chains by thermal currents through microporus compartments [13]. *Yields* of the reaction are low, typically resulting in 30 μmoles of oligomers starting from 10 mmoles of monomers (as determined by ^{31}P NMR titration analysis).

The reaction mechanism. The polymerization mechanism (to be detailed elsewhere) was determined by (i) characterization of the molecular species produced, as determined by MALDI ToF Mass Spectometry; (ii) ^{31}P NMR analysis of the type of phosphodiester bond formed; (iii) analysis of the polymerization products by specific ribonucleases. The ensemble of these analyses shows that polymerization occurs by a simple state-of-art mechanism consisting in the formation of 3′,5′ phosphodiester bonds among stacked cyclic nucleotides.

The reaction is a normal base-stimulated catalysis, occurs in water (Fig. 3, panel A) and in 80% dimethylformamide (not shown), and is strongly stimulated by bases [as determined by the boosting of the reaction with 1,8-Diazabicycloundec-7-ene (DBU), one of the most used non-nucleophilic Lewis base in organic chemistry]. This simple reaction mechanism requires a favorable thermodynamics and the non-fastidious correct positioning of the reacting species, pillared through stacking interactions.

Formamide and polymerization. We have observed that 3′,5′ cGMP solubilized in pure formamide polymerizes following kinetic parameters similar to those observed for polymerization in water (to be detailed elsewhere).

Thermodynamic plausibility. The free energy value for phosphodiester bond formation was calculated to be -5.5 kcal mol^{-1} [115]. The enthalpy of hydrolysis of various 3′,5′- and 2′,5′-cyclic nucleotides was calculated and resulted to be indicative of a high energy bond varying, for different compounds, from -7.7 to -14.1 kcal mol^{-1} [116]. Thus, the coupling between the process of cleavage of the phosphoester bond in cyclic nucleotides and the simultaneous formation of a new bond is thermodynamically favored.

Stacking of guanosine nucleosides. Energetically favorable stacking interactions between bases play an important role in determining and stabilizing nucleic acid structures [117,118]. The physical fundamentals of nucleic acid bases stacking in water have been accurately defined and reviewed [119–121], the thermodynamic parameters for stacking have been determined [122,123].

In ribodinucleoside monophosphates, the stacking preference follows the sequence purine–purine > purine–pyrimidine ⩾ pyrimidine–purine > pyrimidine–pyrimidine [124,125]. In particular, it was established that the stacked states have a 2–6 kcal mol lower free energy than the unstacked states for purine–purine dimers, while for pyrimidine–pyrimidine dimers no appreciable barrier was obtained [124,126]. The most favorable stacking is found in A–A and G–G [120,122,123], determining their rapidly-obtained stacked state in the high dielectric aqueous solution [127]. The stacking–unstacking equilibrium has been characterized as a fundamental parameter in the nick sealing process in DNA [118]. Thus, the polymerization mechanism outlined above is explained by the strong tendency of guanines and of guanosine nucleotides to form stacked, potentially reactive structures.

We wish to note the relevance of stacking forces in the earliest steps of the polymerization processes. As stated in a pioneering work on the conformational properties of nucleosides [128]: "... dimerization of mononucleotides by 3′–5′ phosphodiester formation causes a series of conformational events in a majority of cases and the initiator of these changes is the stacking interaction".

Upon reaction of 3′,5′ cGMP, molecular species were detected by MALDI-ToF Mass Spectometry corresponding to precise multiples of the unitary mass of the cyclic nucleotide = 345 (i.e., 690 for the dimer, 1035 for the trimer, 1380 for the tetramer, and so on) showing the oligomeric stacking of guanosine nucleotides. These forms constitute the initial step of the polymerization reaction. Part of these stacking-organized molecules undergo trans-phosphorylation, affording the observed covalently linked oligomers. Molecular species corresponding to covalently linked dimers, trimers, tetramers, and on, were detected at the expected 708, 1053, 1398, and on.

2.6. Further elongation by terminal ligation

Once formed, oligomers of the size of 2–3 tens of units are bound to rapid degradation in water and do not provide a credible source for the evolution of more complex chemical information. Before even considering the prebiotic relevance of the insertion of these neo-synthesized sequences in a lipid micelle environment and the conditions for their eventual replication, one has to envisage a credible mechanism for their further growth.

A reaction was reported that might provide a partial solution to this evolutionary dead-end: the non-enzymatic terminal ligation of preformed oligomers. This reaction was described for pre-synthesized adenosine oligonucleotides of various lengths [129] and for cytidine–guanosine 24mer oligonucleotides [130]. In the first case, ligation occurred following stacking of the reactants, the second was sequence-complementarity mediated. In both instances >100 nucleotides long molecules were observed [129,130].

The short adenosine oligomers obtained by polymerization of 3′,5′ cAMP through the mechanism described above, undergo spontaneous terminal ligation affording >100 nucleotides-long polymers [31]. The same was observed for the products of polymerization of 3′,5′ cGMP which self-ligated to yield >100 nucleotides long molecules in a slow reaction requiring >50 hrs.

When 3′,5′ cGMP was reacted in water with cytidine oligomers, polymerization of oligoGs was observed growing on the 3′-end of oligo Cs [31,130]. Noteworthy, this terminal polymerization allowed mis-matched growth (that is: not only growth of oligoG on oligoC, but also growth of oligoG on not fully-complementary templating sequences, as CCGCCGCCG [131]).

Whether these self-polymerization mechanisms provide a solution to the onset of development of pre-genetic materials remains to be seen.

3. Synthesis of pre-metabolic components

We have observed that upon heating at 160 °C in the presence of defined catalysts, formamide condenses into carboxylic acids. Namely:

Iron sulphur copper minerals: pyrite FS_2, pyrrhotine FeS, bornite $FeCu_5S_4$ and covellite CuS afford oxalic acid, in various yields [87].

Zirconium minerals: baddeleyte (zirconium dioxide; ZrO_2), cerium zirconium oxide ($CeZrO_4$, as a mixture of ZrO_2 and CeO_2), zircon (zirconium silicate; $ZrSiO_4$), different metal zirconates (lithium zirconate, Li_2ZrO_3; lead zirconate, $PbZrO_3$; barium zirconate, $BaZrO_3$) and zirconium oxinitrate [zirconyl nitrate, $ZrO(NO_3)_2$] afford glyoxylamide, glycolic, lactic, succinic, oxalic, fumaric and maleic acids, in various yields and combinations [89].

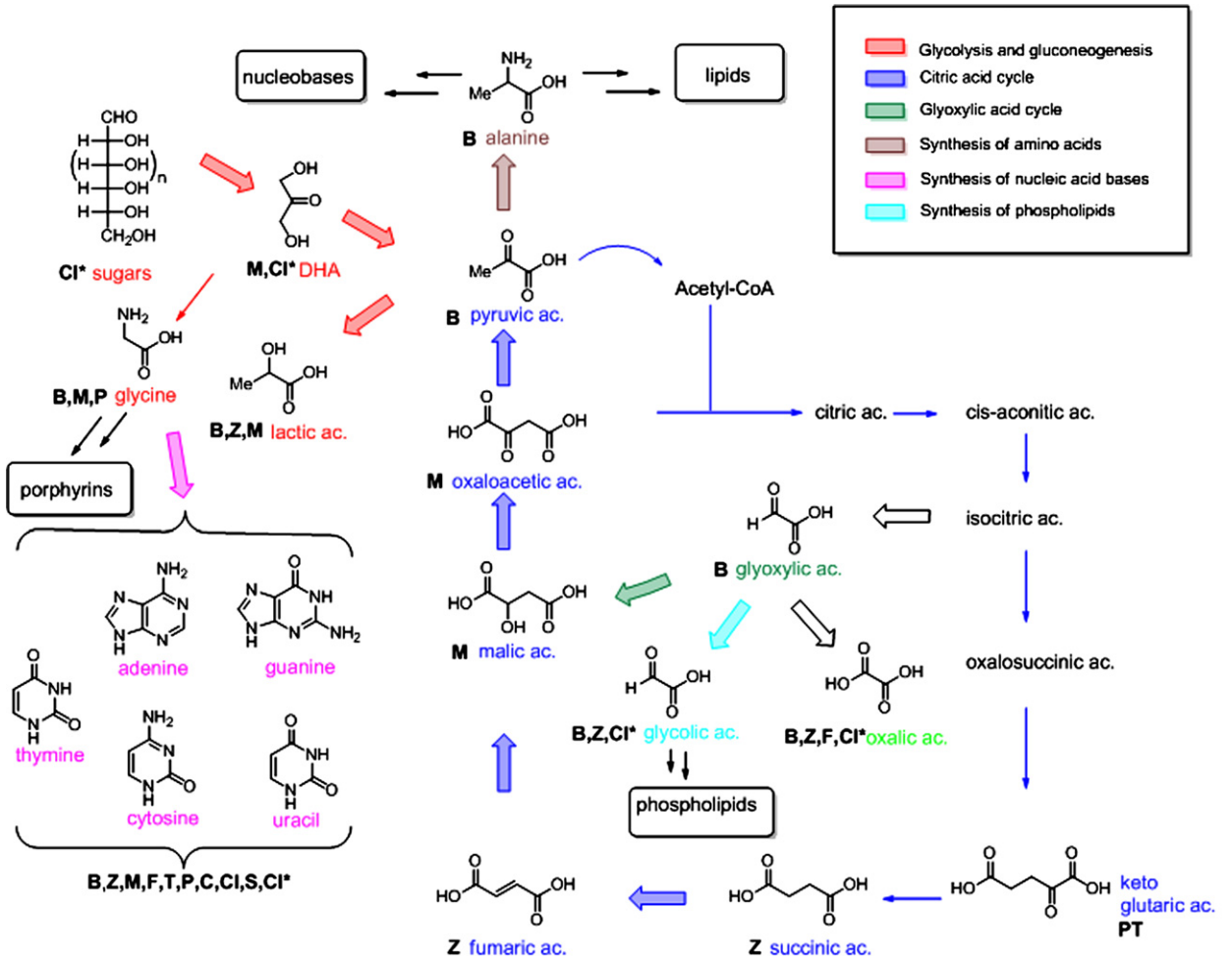


Fig. 4. The major metabolic pathways involving products afforded by abiotic formamide condensation processes. The products abiotically synthesized from formamide are indicated by their name and chemical structure. For a graphical simplification of the scheme, salts are represented as the corresponding acids. Other key intermediates of the pathways that were not obtained in abiotic syntheses from formamide are indicated by their usual name, without chemical structure. Arrows in different colors represent specific metabolic pathways as reported in the inset. Thick arrows start from, or arrive to, products produced from formamide. Thin arrows connect compounds not obtained from formamide. The core of the scheme consists of the citric acid cycle (CAc). The reductive version of this cycle (RCAc) works in the anticlockwise mode. The glyoxylic acid cycle is a variant of the CAc mainly operative in plants. The transformation of glyoxylic acid to oxalic acid is a metabolic cul-de-sac. The symbols in bold at the side of the structure of each compound refer to the mineral(s) or metal oxide(s) required as catalyst for the synthesis of that compound from formamide (see text). The correspondence between acronyms and catalysts is reported. Note that specific compounds can be synthesized from formamide in the presence of different catalysts.

Borates: borax $Na_2B_4O_7{\cdot}10(H_2O)$, colemanite $CaB_3O_4(OH)_3{\cdot}H_2O$, hydroboracite $CaMgB_6O_8(OH)_6{\cdot}3(H_2O)$, kernite $Na_2B_4O_6(OH)_2{\cdot}3(H_2O)$, kurnakovite $MgB_3O_3(OH)_5{\cdot}5(H_2O)$, ulexite $NaCaB_5O_6(OH)_6{\cdot}5(H_2O)$, chambersite $Mn_3B_7O_{13}Cl$, hambergite $Be_2(BO_3)(OH)$, ludwigite $Mg_2Fe_3{+}BO_5$, rhodizite $(K,Cs)Al_4Be_4(B,Be)_{12}O_{28}$, vonsenite $Fe^{22+}Fe^{3+}BO_5$, dravite $NaMg_3Al_6(BO_3)_3Si_6O_{18}(OH)_4$, dumortierite $Al_{6.9}(BO_3)(SiO_4)_3O_{2.5}(OH)_{0.5}$, elbaite $NaLi_{2.5}Al_{6.5}C_3Si_6O_{18}(OH)_4$, kornerupine $(Mg,Fe^{+2})4(Al,Fe^{3+})6(SiO_4,BO_4)_5(O,OH)_2$, axinite-(Mn) $Ca2Mn^{2+}Al_{2}(BO_3)Si_4O_{12}(OH)$, schorl $NaFe^{+2}Al_6(BO_3)_3Si_6O_{18}(OH)_4$, canavesite $Mg_2(CO_3)(HBO_3){\cdot}5(H_2O)$, painite $CaZrB(A_{l9}O_{18})$, boric anhydride B_2O_3, sodium perborate $NaBO_3{\cdot}4(H_2O)$ afford lactic, oxalic, pyruvic and glyoxylic acids, in various yields and combinations [93].

Murchison minerals afford lactic, malic, oxaloacetic and oxalic acids, in various yields [96].

Fig. 4 summarizes these data and lists the carboxylic acids observed as products of formamide condensation, ordered according to their connections in extant metabolic cycles. A large part of the essential components of both the glyoxylic and citric cycles and of gluconeogenesis are observed as products of non-enzymatic, abiotic condensation of formamide.

In a prebiotic setting, cycles of the same reaction are conceivable only through the action of catalysts which, by definition, facilitate the conversion of reactants to products without being themselves changed. In addition, non-enzymatic metabolic cycles should produce their components in sufficient amounts to facilitate an unclogged circular series of transformations. The observed production of the series of carboxylic acids in the presence of a variety of catalysts provides the proof-of-principle that the onset of cycles in a prebiotic environment is conceivable.

The non-enzymatically-driven autocatalytic reverse citric acid cycle RCAc (or: reductive tricarboxylic acid cycle rTCAc) is considered an important driving point in prebiotic scenarios [7,8,132], to the point of being referred to as a universal and possibly primordial metabolic core [8]. This cycle results in the fixation of CO_2 into larger molecules and is initiated by the splitting of citric acid to give acetyl-CoA and oxaloacetic acid. The RCAc is defined as autocatalytic, resulting in two molecules of citric acid starting from one [7,8].

One of the two requirements for this cycle to become autocatalytic, as distinguished from a simple catalytic cycle, is the transformation of pyruvic acid into oxaloacetic acid. The other is the production of CoA-SH. The first is satisfied by the chemistry of formamide, the second involves a component that is too complex to be conveniently considered as prebiotic. The RCAc is shown in Fig. 4 highlighting the compounds abiotically obtained from formamide with the indicated catalysts.

Pyruvic acid connects the RCAc (or the normal citric acid cycle CAc, depending on the clockwise or anticlockwise directions of its rotation) with alanine (the aminoacids branch) and, potentially, with lipids. Pyruvic acid also connects with lactic acid and dihydroxyacetone (DHA), intermediates of glycolysis and gluconeogenesis. Alanine, lactic acid and DHA are all present in the observed panel of compounds. Other key intermediates of glycolysis and gluconeogenesis (namely, glycine and sugars) have also been observed as products of formamide condensation under different experimental conditions. In extant cells, glycine is the only substrate for the synthesis of porphyrins. Moving to other intermediates of the RCAc obtained from formamide, malic acid connects with glyoxylic and isocitric acids in the frame of the glyoxylic acid cycle, a variant of the CAc mainly operative in plants. Glyoxylic acid connects with glycolic acid in the frame of the synthesis of phospholipids, and with oxalic acid in a metabolic cul-de-sac.

The fact that the series pyruvic → oxaloacetic → malic → fumaric → succinic acids is not obtained with a single catalyst tells on one hand that these carboxylic acids may all be synthesized in the frame of formamide chemistry, on the other that probably none of the catalysts involved in the reactions that we have described is a prebiotic reference milestone (at least in the physical–chemical conditions analyzed here) or acted alone.

The series of compounds synthesized from formamide forming the descending branch of the RCAc ends with succinic acid, where CoA-SH merges into the cycle. Acceleration of three of the five reduction reactions on this cycle (oxaloacetic to malic, fumaric to succinic and oxoglutaric to oxalosuccinic acids) occurs abiotically at the surface of the mineral ZnS by photochemistry [133], showing that catalyst-induced transformations may have been instrumental in the establishment of autocatalytic cycles.

Whether autocatalytic cycles have been able to establish themselves is matter of open debate [8,16,17,21,90,133,134]. In particular, the onset of metabolic cycles on the prebiotic Earth has been dubbed as implausible [134], mostly on the basis of kinetic considerations and on the difficulty of justifying both thermodynamically and kinetically the exclusion of side reactions that would disrupt the cycle. This opinion is not generally shared [8,12,14,16,17,21,84–86,132,134,135]. The similarity of spontaneous cycles with metabolic ones has been established for the formose reaction [136–138], which converts formaldehyde to various sugars and other products, and has been suggested for the triose-ammonia reaction [139] and for the acceleration of the HCN oligomerization [140].

The only suggestion made previously of a relationship between HCN chemistry and constituents of the reductive citric cycle is due to Eschenmoser [12] who concludes that a careful analysis of this chemistry may harbour the potential of prebiotic self-organising chemical processes. In particular, the potential generational relationship between HCN and those constituents of the RCAc which are direct precursors of aminoacids in extant metabolism was hypothesized, based on theoretical considerations [12].

Admittedly [12], and as noted [134], there is no robust experimental evidence to support the proposed relationship between HCN chemistry and key metabolic cycles. The data presented here provide such evidence.

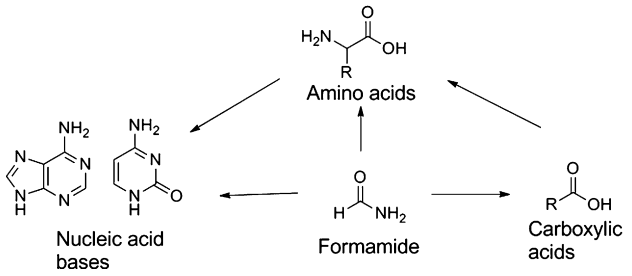


Fig. 5. Interconnections. Formamide is the starting material for the abiotic synthesis of all the pro-biotic nucleic bases, of a spate of carboxylic acids, of the aminoacids glycine and alanine. These compounds are all constituents of extant metabolic pathways.

Beyond this open debate, the observed easy production of groups of serially connected compounds (as oxaloacetic, malic, fumaric and succinic acids; and other acids reported in Fig. 4), and of compounds (as pyruvic acid and alanine) bridging with parts of other important extant cycles, shows that a single chemical frame could have provided a large part of the necessary building blocks. These interconnections are outlined in Fig. 5.

4. The egg *and* the chicken

The top-down reconstruction of the evolutionary process leading to the origin of life rapidly hits paradoxa: how could RNA be synthesized in the absence of RNA polymerase, how could proteins be polymerized in the absence of the ribozymic activities [141,142] of ribosomes? The paradox of the egg and the chicken, clearly stated since two millennia [143] and rephrased in contemporary terms by substituting *egg* with *genotype* and *chicken* with *phenotype*, can only be solved assuming (and showing) a co-evolution of the two systems starting from a common molecular frame. RNA seems to embody this solution [144]. Here is where the top-down reconstruction approach hits the wall of logics: how can energy be reproducibly mastered and made available for the activation of monomers to be polymerized into (pre)genetic polymers in the absence of a metabolism?

A bottom-up experimental approach to the unsolved bias "metabolism-first or genetics-first?" may provide a key to the solution: the two processes started on their evolutionary path together, originated in the same chemical frame.

The formamide system provides chemical plausibility to the onset of key metabolic cycles because the carboxylic acids observed are formed non-fastidiously, in various amounts and combinations, depending on different classes of common catalysts. As for the nucleic acids precursors formed in the same chemistry, the key to the appearance of components of metabolic cycles is the intrinsic synthetic capacity of formamide, not the nature of the catalyst involved.

The fact that different classes of minerals end up producing the same classes of products provides a solution to the topographical conundrum defined by the late L. Orgel "... few would believe that any assembly of minerals on the primitive Earth is likely to have promoted these syntheses in significant yield", and "... the difficulty of finding an ensemble of catalysts that are sufficiently specific to enable the original cycle ..." [134]. The simultaneous synthesis of nucleic bases, carboxylic acids and aminoacids shows that the relevant component of the soup was the reactant, not the catalyst, provided the presence of some of the catalysts mentioned above (or of some combination thereof). Or of one of the certainly numerous alternative catalysts yet to be determined. The decreased relevance of the specificity of the catalyst contributes to the solution of the problem of why and how an ensemble of minerals, each capable to carry on the synthesis of the many different steps of the RCAc, could have been present anywhere on the primitive Earth [145] in order to allow the onset of the universal intermediary metabolism [8].

5. Chemiomimesis

The term *chemiomimesis* refers to a chemical reaction pathway that can be used as a template for the enzymatic processes that will appear later in evolution to yield the same final products [146].

Chemiomimesis thus refers to the possibility that certain biosynthetic pathways reproduce early prebiotic chemistry. The chemical correlations between the condensation of formamide into nucleic bases and the present-day cellular biosynthetic pathways for nucleic acids are remarkable and were discussed in detail [27]. The correlation between the

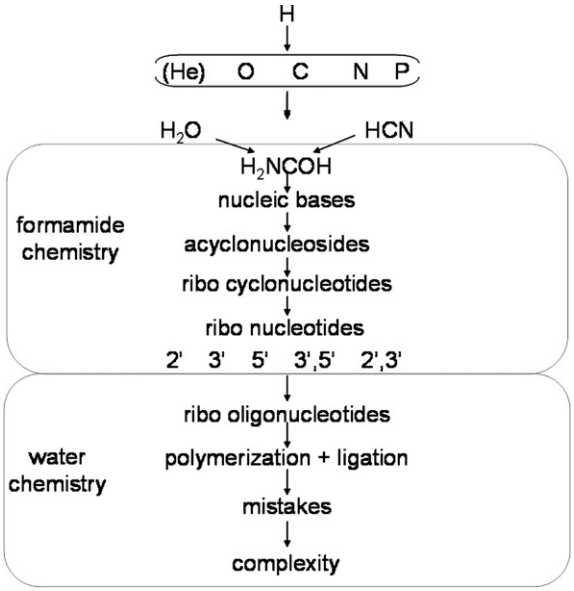


Fig. 6. A flowchart showing from hydrogen to the emergence of pre-genetic sequence complexity. All the first necessary steps may be accomplished in the unitary chemical frame of HCN/formamide chemistry, up to the formation of acyclonucleosides [27] and to the closed-ring phosphorylation of nucleosides [30]. The abiotic synthesis of nucleosides was reported [28,158]. The spontaneous generation of RNA oligomers from purine 3′,5′-cyclic nucleotides [31] and their non-enzymatic ligation in water [129,130] were described. Reprinted from Ref. [131] with modifications.

condensation of formamide into the core components of the key extant metabolic cycles reported above unexpectedly provides the prebiotic scene with chemiomimetic potential for both (pre)genetics and (pre)metabolism.

It was argued that the universal anabolic core was selected by statistical and kinetic factors, not depending on genetics or compartmentation [8]. Similar arguments supported the supposed appearance and evolution of initial simple genomes, seen as generated independently of metabolic contributions, based on intrinsic autocatalytic properties of RNA (or pre-RNA-type molecules) undergoing Darwinian evolution centred on their replication capacity [147]. Both scenarios are based on solid chemical theories and on experimental observations. Removing the reciprocal supposed independence and assuming a potential coexistence in the same chemical frame, the way to experiment the possibility and the benefits of the cooperation of the two systems is open.

6. Conclusions

The most abundant organic (HCN) and inorganic (H_2O) combinations of the four most frequent atoms of the Universe H, C, O, N (www.astrochemistry.net) react to yield formamide H_2NCHO. Formamide has the peculiar property of being liquid between 4 and 210 °C, thus being easily concentrated from dilute aqueous solution above 100 °C and available for reaction in numerous environments.

The content of this article describes how all the steps are plausible from the initial syntheses of nucleobases to RNA in the frame of reference of formamide first, then in water.

6.1. (Pre)genetics and (pre)metabolism

HCN/formamide chemistry provides a plausible unitary frame for the origin of the genetic polymers in the extant forms of life on planet Earth. Formamide condensation affords all the necessary nucleic bases and acyclic nucleosides [26,27,29], allows the efficient phosphorylation of nucleosides in every possible position of ribose [30], also yielding cyclic phosphate forms. In water, purine 3′,5′ cyclic nucleotides polymerize to oligomers [31] which may non-enzymatically ligate affording long RNA molecules [129,130]. A schematic view of this series of connected possibilities is reported in Fig. 6.

This path to the spontaneous generation of chemical complexity highlights the ability of RNA to potentially solve the problem of the origin of genetic materials, based on its intrinsic catalytic properties and on the attainment of increasingly higher levels of molecular stability and complexity [148].

From the one-carbon atom formamide the RNA precursors are simply obtained by warming at 160 °C [80,96] or by decreasing the temperature while providing part of the energy in the form of UV radiation [81,82]. These synthetic reactions occur in the presence of any one of the following common terrestrial minerals: calcium carbonate, zeolite, alumina, titanium oxides, clays, olivines, phosphates (reviewed in [27,80]), and of iron-sulphur–copper minerals [87], zirconium minerals [89], borates [93]; or, from outer environments, in the presence of Murchison meteorite minerals [96]. The generality of the catalysts involved indicates the non-fastidious nature of the synthetic process of nucleic acids precursors based on formamide chemistry.

The same spontaneity and the same non-fastidious requirement for specific catalysts are observed for the synthesis of a large number of connected carboxylic acids in the core of the anabolic processes, as detailed in Section 3. At this point the prebiotic potentiality of this chemical system delineates as a credible possibility.

We do not venture into hypotheses on what ignited the autocatalytic hypercycle that led to life. Nevertheless, if one considers life as a hypercycle of the nucleic acids' and of the carboxylic acids cycles, one at least knows where to look for it. A brief discussion on the "definition of life" may help in delimiting this argument.

Definition of life is an open problem. The current status of the definition problem is discussed in-depth in a recently appeared collection of essays [149]. No definition is generally and univocally accepted. Interestingly, a critical analysis of the over 100 definitions of life existing today [150,151] defines a consensus. The analysis of the terms most frequently occurring in these definitions results in the concise formulation: life is self-reproduction with variations [152]. Analytical comparison with the most frequently used definition ("life is a self-sustained chemical system able of undergoing Darwinian evolution" [1]) reveals that the similarity is only apparent. The only common term in the two definitions is "self", which appropriately highlights the spontaneous character of the process.

Our operational opinion on this matter is that life is (i) a process rather than a system, and that (ii) variation is the most deeply rooted intrinsic character of nucleic acids. The nucleotide sequence of a given nucleic acid molecule is bound to change because of the intrinsic chemical properties of the sequence replication process. This can be brought to experimental analysis in sequence complementarity-driven abiotic RNA replication [130] in a system consisting of sequence-complementary tail polymerization of 3′,5′ cGMP on a polyC template. The system allows one round of replication and accommodates sequence mistakes. As pointed out [153], any experimental work involving the GCCn*GGCn replicator would border the life-non life transition.

The nature of the very first peptides has been indicated and the reasons for the assignment have been presented [154,155]. These very first peptides supposedly were $(Ala)_7$ and $(Gly)_7$, encoded by $(GCC)_7$ and $(GGC)_7$, the two strands of the earliest RNA duplex genes [154,155]. Hence the relevance of GCCn*GGCn replication studies. In this perspective the precise replication of the protogenic sequence represents the establishment of order, in informational terms. Its unprogrammed variation represents disorder. Entering variability and evolution in this replication scheme means considering the passage from disorder to order, from non-life to life [153].

An extreme definition of life is provided by Emile Cioran: "La vie est le kitsch de la matière" [156]. Even without recurring to such an extreme view, this pessimism is instrumental in making the point: life is nothing special, its manifestations are all well within the laws of thermodynamics. What distinguishes it from more elegant simple processes as atomic transformations or crystallizations is complexity, resulting from the interaction among different sets of reactions and, eventually, their mutual interference. These reactions segregate information and anabolic products, for a period. From a minimalist point of view this sort of complexity can be easily considered as kitsch. Especially so because the more the system is complex, the less it is foreseeable and controllable. Evolution is not foreseeable nor controllable and, according to Trifonov's view, evolution is one of the pillars of the definitions of life [152].

The simple chemistry illustrated here is inspired by the John Occam's razors logics ("the simplest is the likeliest") and by Victor Stenger's aphorism "Something came from nothing because it was more stable than nothing" [157], embodying in a prebiotic perspective the second principle of thermodynamics.

Acknowledgements

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