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Determinism of formamide-based biogenic prebiotic reactions

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A B S T R A C T

Formamide reacted in the presence of a catalyst and of a source of energy affords a rich and complex panel of compounds, including amino acids, amino sugars, nucleic bases, nucleosides, carboxylic acids, aliphatic chains, and more. Nor the source of energy nor the type of catalyst are fastidious. All the catalysts tested have activity; each catalyst affords its own specific set of products, although the panels of products of each catalyst largely overlap. Potentially biogenic compounds form in reasonable conditions and the chemistry that determines the initial syntheses is facile. Hence, Darwins *warm little pond* did not rely on exotic environments nor on magic tricks. The type of molecules resulting from a mixture of formamide and of two selected products of its initial reactions hint that the initial prebiotic soup was deterministic and oriented towards life-as-we-know-it.

1. Introduction

We published in 2012 the article "Formamide and the origin of life" [1], describing the reactions which occur when formamide is reacted in the presence of a catalyst and of a source of energy. The panel of catalyst analysed in separate experiments consisted in 9 metal oxides, carbonates, volcanism-related minerals, clays, phosphates, Fe/S/Cu/Zr/B-containing minerals, circumstellar/cometal dusts analogues, Murchison meteorite; the energy source applied was thermal.

Looking in perspective, the list of catalysts analysed was large but not exhaustive, the energy form was strongly biased. However, even in those limited set-up of analysed conditions, formamide proved its worth: the panel of compounds that formed was rich and complex. The reactions afforded amino acids, amino sugars, nucleic bases, acyclonucleosides, carboxylic acids, aliphatic chains, and more. The conclusion was inescapable: *potentially biogenic compounds could form in reasonable conditions and the chemistry that determined the initial syntheses was facile*. Hence, Darwin warm little pond [2] did not rely on exotic environments nor on magic tricks.

Other conclusions were unavoidable: (a) each catalyst afforded its own specific set of products; (b) all the catalysts tested had activity; (c) the panels of products largely overlapped. Hence, the key actor of the initial biogenic chemistry was formamide and the set of reactions was determined by the catalyst-imposed chemistry.

The first conclusion makes a posteriori sense: life-as-we-know-it is made of Hydrogen, Oxigen, Carbon and Nitrogen; the other elements (Phosphorus, Sulphur and Iron, for a start, but also Magnesium, Manganese, and others) are important but specialized and ancillary. Formamide (NH_2COH) contains the four of them.

H, O, C and N are the most abundant elements of the universe [3]. If life, as we deem, is nothing else than a series of cyclic reactions which reproduce themselves under the blueprint guidance of codes, it is reasonably made of the most commonly available materials. This property of life accommodates both the operative definition due to Edward Trifonov ("*life is reproduction with variations*") [4], and the vision of life as form of chemical-physical topology that owns much to Eörs Szathmary [5]. The definition by Trifonov provides the consensual definition accepted by contemporary science, as it was obtained by carrying out a comparative structuralistic analysis of the 123 existing definitions of the word "life", so reaching the underlying consensus. In this perspective, life is the interplay of cycles,

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whose wheels turn according to the information settled in their history and by the environments they have gone through. The first interplay in the combinatorial game that has given rise to the cycles could not have occurred among rare and marginal types of atoms, for economical and systemic reasons. Other elements entered the little pond later, providing specialized functions and optimization to robust and established cycle(s), whose nature is still elusive. Nevertheless, the experiments reported in [1] showed that the start was easy, and hinted that the first contacts could have been between formamide and any one of a large set of minerals. The "variations" invoked in the definition by Trifonov are a necessary attribute of a chemical system which must continuously adjust to a continuously varying supporting environment. This requirement accommodates the "Red Queen principle", as described by Lewis Carroll [6]. The Eörs Szathmary's cycles require the establishment and function of codes.

The properties of nucleic acids master chemistry and structural rules, and create emergent properties as topology. Proteins have comparably vast potentialities, but different. In the moment in which one class of molecules, the nucleic acids in our case, finds the way of inducing and controlling another class of macromolecules, the proteins in our case, in the moment when it learns how to guide them to specific actions, then the limits of the properties of one specific class of macromolecules are by-passed. Codes for controlled interaction allow that the properties of each class do not only add, they rather multiply. Codes allow cycles to create the potential for establishing and reproducing themselves.

The second conclusion ("*all catalysts tested had activity*"; *hence, the key actor of the initial biogenic chemistry was formamide*) has two implications: the potentiality of formamide as initial building block and the relevance of exploring all sorts of catalysts. This exploration has been carried out (see below) and it has provided confirmation of the initial conclusion: all minerals have catalytic activity. Hence, the key player in the earliest syntheses was actually formamide.

2. Formamide

Hydrogen, carbon and nitrogen combine in hydrogen cyanide (HCN), which, in the inter- and circum-stellar space, is the most abundant molecule among those with three atoms containing a Carbon [3]. Other different three-component molecules with the same composition are there in lower amounts, or are absent. The most abundant three atoms molecule containing O and not C is water [3]. Reaction of HCN with H_2O affords NH_2COH formamide, which is thus a stabilization form of the energy initially contained in HCN. Formamide is present in vast amounts in space [7]. The origin of the large clouds of formamide present in space is under debate [8]. The hypothesis that formamide formed in the shocking events at the beginning of star formation has been formulated [9].

The chemistry of HCN as potential initial of prebiotic chemistry was thoroughly explored [10,11] and it was shown to be very fertile [12]. From the initial observation of the synthesis of adenine [13] to more recent reports [10,11,14], it has shown its capacity, especially so in the emergence of genetic polymers and proto-metabolism [15].

In addition to HCN, other one-carbon atom precursors maybe active components of the initial syntheses, and provide specialized chemistries, parallel to that of formamide. The most relevant ones are formaldehyde CH_2O [16], methanol CH_3OH [17], ammonium formate NH_4+ $HCOO-$ and formic acid HCOOH [18]. These 1C-atom compounds are easily interconverted, and the presence of one or the other depends on the conditions. The formation of formamide from formic acid and ammonia depends on the temperature [19], and formamide was observed in the boro-silicate glass container [20] during the Miller-Urey experiment [21].

From the prebiotic point of view, HCN and NH_2COH are not mutually exclusive. Relevant disadvantages of HCN are its lack of propensity to accumulation, and its extreme reactivity causing instability of its products. HCN-based syntheses require the assistance of an operator for interventions, purifications and separations. This is not the case for the much more stable formamide, which has already quenched with water, or never acquired, or has somehow lost, its hyper-reactivity during one of the many pathways that can lead to its formation [1]. Given its stability and its boiling point of 204 °C, formamide can easily accumulate from water solutions, and provides easy ways to further levels of chemical complexity. These ways were explored.

3. Formamide-based syntheses

3.1. Space-wise

Meteorites have been identified as efficient catalysts for the conversion of formamide to a large panel of organic compounds with biological relevance under thermal conditions [22]. Among them, the complete set of RNA's nucleobases, carboxylic acids that are intermediates of the Kreb cycle and several proteinogenic and un-proteinogenic amino acids. Meteorites of the chondrite type, which were common in the original composition of the Solar System, were catalysts more efficient than iron, stony-iron and achondrite meteorites. The syntheses worked in pure formamide and formamide/water mixture, including sea water [23] and thermal water from Phlegrean Fields (Italy) [24]. The analysis of the source of energy allowing the prebiotic reactions of formamide was extended to high energy proton beams modelling the solar wind [25], obtaining an extremely rich, variegate, and prebiotically relevant panel of compounds, which include the complete set of natural RNA and DNA nucleobases and 4 nucleosides in which the nucleobase is bonded to sugar with the naturally occurring regio-chemistry and stereo-chemistry (that is : beta-nucleosides and N-9 and N-1 substituted purines and pyrimidines, respectively). The reaction pathway for the synthesis of nucleosides from formamide was analyzed by experimental and computational approaches, and the thermodynamic control in the addition of the nucleobase and sugar, as well as the role played by the mineral surface in the stereo-selectivity of the formation of beta-isomers, was discussed in detail [26,27].

The one-pot synthesis of nucleosides from a prebiotic precursor as simple as formamide was unprecedented [28]. Alternative pathways have been reported. They include multi-step syntheses for the construction of the nucleobase on a pre-formed sugar [29], or the construction of the sugar on a pre-formed nucleobase [30]. Meteorites and high-energy proton beams were able to trigger the

trans-glycosylation process between adenosine and other purine and pyrimidine nucleobases, and further extended the prebiotic worth of the reaction to the synthesis of thymidine, 2′-deoxyadenosine and cytidine [31]. In addition, adenosine was phosphorylated under similar experimental conditions to yield the corresponding nucleotides (including cyclic nucleotides monophosphate) in the presence of both inorganic phosphate and of minerals containing phosphates [32]. Adenosine 5′, 3′-mono-phosphate was polymerized to small RNA molecules by a novel un-template click-like condensation in which formamide played the role of organo-catalyst [33]. Nucleobases, amino acids and carboxylic acids were also obtained from formamide and meteorites under heavy ion irradiation, extending the possibility of the reaction to cosmic rays [34].

The prevailing catalytic role of meteorites in formamide prebiotic chemistry [35] suggests a unifying concept for the synthesis of nucleobases [36], nucleotides, oligonucleotides [37] and small RNA [38]. In this scenario, formamide and meteorites were also involved in the evaluation of the Primordial Multifunctional organic Entity (PriME) model, by analyzing the hitherto overlooked structural and physicochemical similarities between the Insoluble Organic Matter (IOM) in meteorites and the dark, insoluble, multifunctional and radio protective melanin polymers in Earth [39]. In the PriME scenario, IOM and polycyclic aromatic hydrocarbons (PAH) may have played a key role in three critical processes for the origin of life: a) metabolism and accumulation of molecules; b) catalysis; and c) protection of molecules against radiation and successive compartmentalization. In this context, the high-energy proton-beam irradiation of representative naphthols and meteorites, in the presence of urea or formamide, promoted the synthesis of prebiotically relevant intermediates of oxyPAH and nitrogenPAH, expanding the range of intermediate reactive species to include, among others, quinones, as a possible contributory mechanism for the formation and processing of IOM in meteorites [40].

3.2. Earth-wise

The formamide prebiotic chemistry was studied in terrestrial and planetary geochemical scenarios typical of the primitive Earth, such as serpentinization processes. Silicates and metal oxides increased the chemical complexity by catalyzing the conversion of formamide into a large panel of biomolecules, including nucleobases and analogues, amino acids, sugars and carboxylic acids [41]. The relevance of these reactions was associated to the supramolecular character of the overall process, including the ordered structures of chemical gardens [42] and of silica metal oxide vesicles [43]. In these latter cases, due to the high alkaline properties of the saturated silica produced from serpentinization, silicate can precipitate from the bulk of the solution to yield asymmetric membranes bearing the highest concentration of metal ions inside of the growing silicate structure. The breaking of symmetry produces chemical energy for the condensation of formamide to biomolecules, as well as un-precedented catalytic compartmentalization, since the nucleobases are selectively synthesized inside the membrane, while amino acids and carboxylic acids prevailed out-side. It should not escape notice that this compartmentalization effect is similar to the molecular organization in the modern cell, highlighting the possible role of chemo-mimesis in Darwinian molecular evolution [44]. Chemo-mimesis is a powerful concept to understand the mechanisms for the passages from the abiotic to the biotic [45], indicating that processes characterizing biological phenomena often have purely abiotic precedents [46].

Silicates catalyzed the prebiotic synthesis of biomolecules from formamide also in more complex systems, including gas-phase reactions. For example, the role played by borosilicate in the variability and selectivity of the Urey-Miller reaction was shown by comparison of the efficacy of the process using an inert reactor made from Teflon as reference. When the reaction was run in Teflon, a very limited number of reaction products was isolated. In the original Miller glass container, borosilicate was released in the bulk of the reaction during the electric discharge as a consequence of the increase of the pH value. Borosilicate concentrated at the bottom of the flask acting as catalyst for the condensation of formamide and of HCN oligomers into a very large panel of reaction products. In the absence of borosilicate, the catalytic effect was lost, and only few compounds were synthesized [47].

The variety of reaction products obtained from formamide is shown in Table 1. The products are reported on the basis of the chemical structure in the following representative groups: a) pyrimidines and analogues; b) purines and analogues; c) nucleosides and nucleotides; d) heterocycles and sugars; e) amino acids (both proteinogenic and un-proteinogenic); f) carboxylic acids. The number and variety of reaction products is impressive. A number code is provided below the chemical structure in order to correlate the synthesis of a specific compound to the nature of the mineral catalyst and of the energy source applied in the transformation. The combinatorial explosion of reaction products of biological relevance suggests the possibility to synthesize at the same time all the key intermediates required for the pre-genetic and proto-metabolic apparatuses.

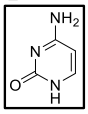
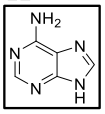
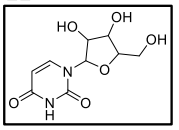
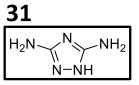
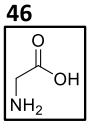
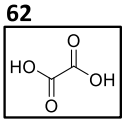
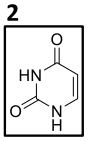
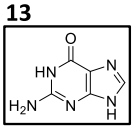
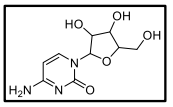
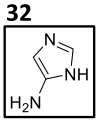
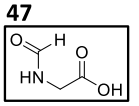
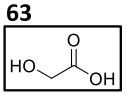
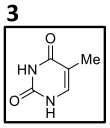
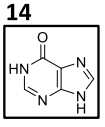
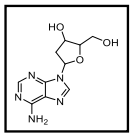
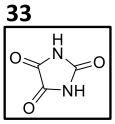
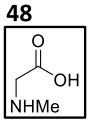
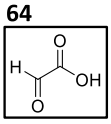
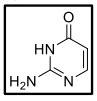
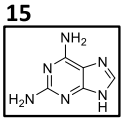
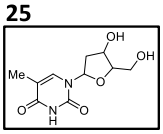
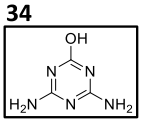
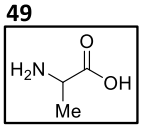
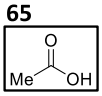
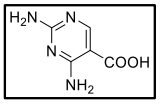
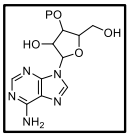
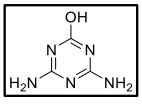
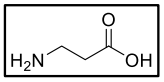
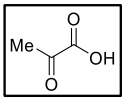
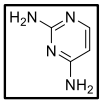
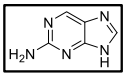
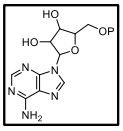
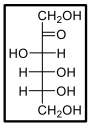
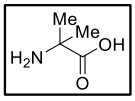
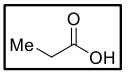
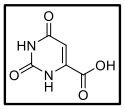
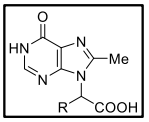
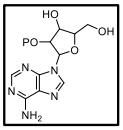
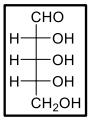
Purine and pyrimidine nucleobases are synthesized with the highest frequency, followed by the most simple amino acids and carboxylic acids.

4. Directing the reactions towards biogenesis

The word “initial”, when referred to the Darwin warm little pond has no real meaning. We know the end-result (that is: what we dub “life”), consisting in repeated code-controlled cycles of information-bearing macromolecules. We also start having an idea of the whereabouts of the initial production of biogenic bricks. Summarizing this point: in a precursor pool made of interconvertible information-bearing, three/six-atoms, one-carbon containing compounds (formaldehyde CH_2O, methanol CH_3OH, ammonium formiate NH_4+ $HCOO-$, formic acid HCOOH, hydrogen cyanide HCN) pool, reactions occurred which afforded molecules with larger complexity and higher stability. The number and variety of these molecules is large but limited, necessarily within the rules of combinatorial chemistry. The system is easy and fertile; it provides many possibilities, potentially too many.

Our analyses were devoted to the most productive system, the one based on formamide, and showed that the pool of products, obtained when it was reacted in the presence of one of many different catalysts and was triggered by one of many forms of energy, is

Table 1
Variety and structural motif of the reaction products obtained from formamide and formamide derivatives in the presence of minerals and different energy sources.

Pyrimidines and analogues	Purines and analogues	Nucleosides and Nucleotides	Heterocycle and sugars	Aminoacids	Carboxylic acids
1  I, X, XVI, XVII, XVIII, XIX	**12**  I, VII, VIII, IX, XVI, XVII, XVIII, XIX	**22**  IX	**31**  XVIII, XIX,	**46**  I, VII, VIII, X,	**62**  I, VII, VIII, X,
2  I, VII, VIII, IX, XVI, XVII, XVIII, XIX	**13**  II, VII, VIII, XI, XVI, XVIII, XIX	**23**  XII,	**32**  IX	**47**  I, VII, VIII, X,	**63**  III, VII, XA,
3  IX, XIX	**14**  III, VII, VIII, X, XVI, XVIII, XIX	**24**  XII	**33**  VI, XVIII, XIX,	**48**  III, VII,	**64**  I, VIII,
4  I, VII, VIII, X, XVI, XVII, XVIII	**15**  VII, XI, XVI	**25**  XII	**34**  VII, XVII, XVIII, XIX,	**49**  I, X,	**65**  VI,
5  XVII	**16**  I, VIII, XI, XVIII	**26**  XV	**35**  VII, XVII, XVIII, XIX,	**50**  I	**66**  I, VII, VIII, X,
6  XVII	**17**  IV, II	**27**  XV	**36**  VIII,	**51**  III, X	**67**  III
7  XII, XVI	**18** 	**28** 	**37** 	**52** V	**68** I, VII, VIII, X

(*continued on next page*)

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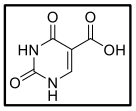
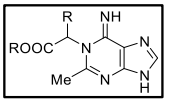
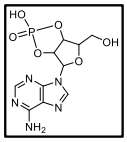
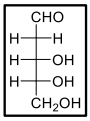
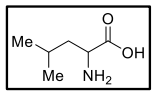
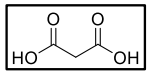
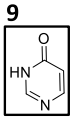
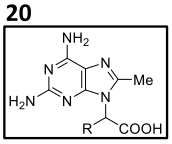
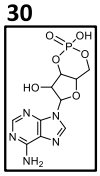
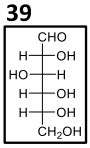
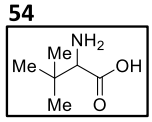
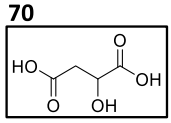
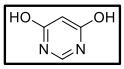
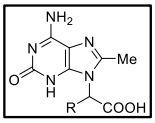
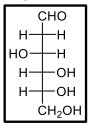
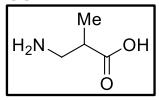
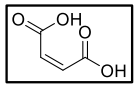
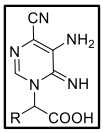
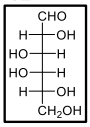
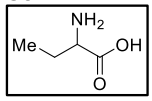
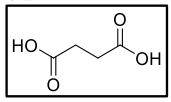
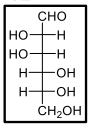
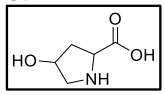
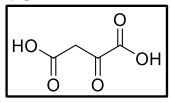
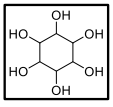
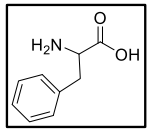
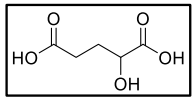
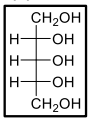
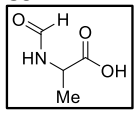
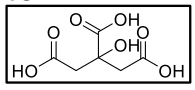
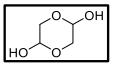
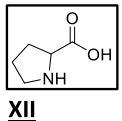
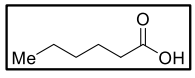
8  **VII**, **XVIII**	**19**  **XXI**	**29**  **XV**	**38**  **V**	**53**  **III**	**69**  **IIIA**, **VIII**, **XA**
9  **I**, **VII**, **VIII**, **XI**, **XVII**, **XVIII**	**20**  **XXIII**	**30**  **XV**	**39**  **XII**	**54**  **VI**	**70**  **IIIB**, **VII**, **VIII**
10  **XIII**	**21**  **XXIV**		**40**  **V**	**55**  **I**	71  **IIIA**, **VII**,
11  **XXII**			**41**  **VA**	**56**  **IV**	**72**  **IIIA**, **VII**, **IX**
			42  **VA**	**57**  **IV**	**73**  **I**, **VII**, **X**
			43  **XII**	**58**  **III**	**74**  **I**, **VII**, **XII**
			44  **XII**	**59**  **X**	**75**  **VII**, **XB**
			45  **XVII**	**60**  **XII**	**76**  **IIIA**, **XIII**

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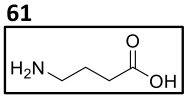
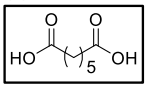
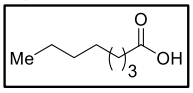
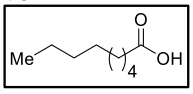
Table 1 (*continued*)

				61  **XIIIA**	**77**  **IV**, **XII**
					78  **VA**, **XIII**
					79  **V**, **XII**

I: Iron, Stony iron, achondrite and chondrite and heat; II: achondrite and heat; III: Iron and Stony iron and heat; IIIA: Iron, stony iron and chondrite and heat; IIIB: Iron, stony iron and achondrite and heat; IV: Stony iron and heat; V: Stony iron, achondrite and chondrite and heat; VA: Chondrite, achondrite and heat; VI: Chondrite and heat; VII: Chondrite and sea water and heat; VIII: Stony iron, achondrite, chondrite and thermal water and heat; IX: Stony iron, achondrite and chondrite and proton beam; X: Iron, stony iron, achondrite and chondrite and proton beam; XA: Iron, stony iron, and achondrite and proton beam; XB: Iron, stony iron, and chondrite and proton beam; XI: Achondrite and chondrite and proton beam; XII: Chondrite and proton beam; XIII: Iron and proton beam; XIIIA: Achondrite and proton beam; XIV: Stony iron and proton beam; XV: Adenosine, chondrite, formamide and phosphorus source, and proton beam; XVI: Stony iron and chondrite and heavy atoms; XVII: chemical gardens and heat; XVIII: Silica metal vesicles and heat; XIX: Urey-Miller conditions. XX: Multicomponent reaction between AMN, amino acids, trimethyl orthoacetate and formic acid; XXI: Multicomponent reaction between DAMN, amino acids, trimethyl orthoacetate under photo-thermal conditions; XXII: Multicomponent reaction between DAMN, amino acids, trimethyl orthoacetate under photochemical conditions; XXIII: Multicomponent reaction between AMN, amino acids, trimethyl orthoacetate and guanidine; XXIV: Multicomponent reaction between AMN, amino acids, trimethyl orthoacetate and urea. Pyrimidines and analogues: (**1**) cytosine; (**2**) uracil; (**3**) thymine; (**4**) isocytosine; (**5**) 5-carboxy-2,4-diaminopyrimidine; (**6**) 2,-diaminopyrimidine; (**7**) orotic acid; (**8**) 5-hydroxymethyl uracil; (**9**)pyrimidin-4(3H)-one; (**10**) 4,6 dihydroxypyrimidine; (**11**) *N*-1 pyrimidine derivatives; (**12**) adenine; (**13**) guanine; (**14**) hypoxanthine; (**15**) 2,4-diaminopurine; (**16**) purine; (**17**) C-2 aminopurine; (**18**) *N*-1 aminopurines; (**19**) *N*-1 purines; (**20**) *N*-9 diaminopurines; (**21**) *N*-9 guanines; (**22**) uridine; (**23**) cytidine; (**24**) 2′ -deoxyadenosine; (**25**) 5-methyl-2′deoxyadenosine; (**26**) adenosine 3′monophosphate; (**27**) adenosine 5′monophosphate; (**28**) adenosine 2′monophosphate; (**29**) adenosine 2′,5′-cyclic monophosphate; (**30**) 3′,5′ adenosine- cyclic monophosphate; (**31**) 2,4-diaminotriazole; (**32**) 2-aminoimidazole; (**33**) chetohydantoin; (**34**) ammelide; (**35**) 4-idroxy-2,6-diaminotriazine; (**36**) d-fructose; (**37**) ribose; (**38**) deoxyribose; (**39**) d-glucose; (**40**) d-2′-deoxy glucose; (**41**) d-galactose; (**42**) d-mannose; (**43**) arabitol; (44); (**45**) 2,4-dihydroxydiossane; (**46**) glycine; (**47**) formyl glycine; (**48**) N-methyl glycine; (**49**) alanine; (**50**) beta-alanine; (**51**) alpha-methyl alanine; (**52**) valine; (**53**) leucine; (**54**) 2-amino-3,3-dimethylbutanoic acid; (**55**) beta-amino-isobutyric acid; (**56**) 2-aminobutanoic; (**57**) 4-hydroxyproline; (**58**) phenylalanine; (**59**) N-formyl alanine; (**60**) proline; (**61**) γ aminobutyric acid; (**62**) oxalic acid; (**63**) glyoxylic acid; (**64**) glycolic acid; (**65**) acetic acid; (**66**) pyruvic acid; (**67**) propionic acid; (**68**) lactic acid; (**69**) malonic acid; (**70**) 2-hydroxysuccinic acid; (**71**) maleic acid; (**72**) succinic acid; (**73**) 2-oxosuccinic acid; (**74**) 2-hydroxypentanedioic acid; (**75**) 2-hydroxypropane-1,2,3-tricarboxylic acid; (**76**) hexanoic acid; (**77**) heptanoic acid; (**78**) octanoic acid; (**79**) nonanoic acid.

large and complex. This resulting complex ensemble of molecules could have been directed to several possible lateral pathways, not necessarily resulting in the type of cycles that we know.

5. Too many products

The aim of these studies is the verification of the biogenicity of the formamide-based reactive pool. Thus, we have explored the possibility that providing to the pool a second level of information we could direct the successive syntheses in the direction of life as we know it.

In this frame, it is worth noting the combinatorial explosion of reaction products obtainable from mixtures containing more than one prebiotic precursors, mimicking more realistic multi-component processes [48]. Urea, guanidine and formic acid are easily produced in situ from formamide under a large variety of experimental conditions. Once produced, they can combine among themselves or, in alternative, they can combine with other prebiotically ubiquitous compounds, such as HCN oligomers, aminomalonitrile (AMN) and diaminomaleonitrile (DAMN), inducing the increase of the chemical complexity of the system. On this regard, AMN and DAMN are produced from formamide under thermal shock and high-energy conditions [49], performing as reactive intermediates in the one-pot synthesis of canonical and unusual nucleobases and nucleosides by ionic and radical mechanisms [50]. The multicomponent condensation of AMN with amino acids and trimethyl orthoacetate, assisted by microwave irradiation and successive reaction with formic acid, yielded a large panel of purine derivatives decorated in the N-9 position of the heterocyclic ring with amino acid

side-chain (Table 1; column purine and analogues) [51]. These compounds are building blocks of RNA-peptide chimeras, which may have co-evolved with RNA during molecular evolution playing the double role of storage of information and molecular catalysis [52]. As an alternative, purine analogues substituted in N-1 position of the heterocyclic ring with amino acid residues were synthesized by the multicomponent condensation of DAMN, trimethyl orthoacetate and amino acids under photo-thermal conditions (Table 1; column purine and analogues) [53]. Noteworthy, the regio-selectivity of the multicomponent reaction was controlled by the energy source used for triggering the condensation, and N-1 substituted pyrimidine derivatives were obtained repeating the reaction in the presence of UV-photons as the only energy source of the process (Table 1; column pyrimidines and analogues). The analysis of the prebiotic multicomponent reaction was extended to the preparation of 2, 6-diamino-adenine derivatives decorated in N-9 position with amino acid residues by thermal condensation of AMN, amino acids and trimethyl orthoacetate in the presence of guanidine (Table 1: column purines and analogues) [54]. In a similar way, isoguanosine derivatives decorated in N-9 position with amino acid residues were obtained from AMN, amino acids and trimethyl orthoacetate in the presence of urea.

The pool of products obtained from the contemporaneous presence of formamide, AMN and DAMN, and one amino acid, encompasses *all* the biological nucleic bases, several amino acids, RNA-peptide chimaeras, carboxylic acids, and two amino acids-long peptides. Noteworthy, the presence of RNA-peptide chimaeras functionalized in different positions of the heterocyclic ring points to possible mechanisms for the evolution of alternative (pre)genetic codes. Formamide is present in large clouds in the universe, amino acids are normally observed as product of formamide or HCN condensation, AMN and DAMN are HCN oligomers. Hence, this mixture is a sub-set of a largely possible general universal condition; it is not an advanced stage towards complexity: all the starting components are only the second step of facile condensation reactions. Thus, the reaction can be deemed as genuinely prebiotic. The contemporary presence of these compounds is sufficient to favour the biogenic direction. Given that most of the relevant compounds were there since the very first reactions, these results point to the biogenicity of the HCN-formamide chemistry and to its conservativeness. The system reveals the conservativeness of the origin-of-life in general (most of the relevant compounds were there since the very first reactions), and to the fertility of the HCN-formamide chemistry (the large variety of biogenic compounds formed one-pot in a single condition). The interest of this research is that, in this system, the pool of molecules formed is quite close to biogenicity.

6. Easy reactions

If one-carbon containing compounds are interconvertible under simple environmental conditions, if every mineral tested behaves as catalyst, if the source of energy is not particularly demanding, these reactions are "easy". A binding conclusion of the exploration of the composition of the pool of products of these easily occurring reactions consists on the realization that the basic components of what we consider "living" are the necessary outcome of the combinatorial chemistry of Carbon. The direction taken by the successive interactions of the first-level of products in multicomponent processes is determined by the presence of nucleic acids and of protein precursors to yield hybrid and chimera molecules able to improve further acceleration of molecular evolution events. Molecules can interact, they can improve organo-catalysis, finally breaking the temporal symmetry and generating the emergence of dissipative structures resembling living organisms.

7. Molecular determinism

The result consisting in the observed facility of synthesis and in the nature and complexity of products obtained from formamide under a large variety of catalysts and of energy sources entails two ancillary conclusions.

The first conclusion derives from the observation of the very composition of the pool of compounds: all the precursors of RNA are present in the same pot, along with numerous amino acids and with nucleic base-amino acid chimeras [53-55]. We have hinted above to the relevance of the coordination of the properties of different class of macromolecules, obtainable through codes. We know that such functional interaction has occurred and made possible by the evolution and establishment of the "genetic" code. The control acquired through the genetic code has provided the nucleic acids with the ability to master the properties of proteins, to use them for their own purposes of reproduction and transmission of information, to establish cycles.

The words "nucleic acids" and "proteins" translate, after biogenesis, to "genotype" and "phenotype" (which in living entities also includes carboxylic acids and membranes, whose precursors are present in the formamide-derived pool). With no phenotype, the genotype would have not transmitted itself; with no genotype, the phenotype would have succumbed to the laws of disorder and to the domination by local conditions. Hence, the necessity of the concurrent and cooperative evolution of both nucleic acids and proteins, all contained in membrane-defined spaces where concentrations and evolution, could take place. The shaping and the interaction of genotype and phenotype are the ultimate form of stabilization of the components of the pool. Stabilization is the Darwinian advantage that drove their evolution.

That is *almost* to say that the basic structure of LUCA was already there since the beginning, determined by the very composition of the rich pool of easily obtained compounds, which organized themselves in the only way they could. Spelling it out more bluntly: the structure of LUCA was already there, determined by the combinatorial chemistry of Carbon. Not much of what shows up in Table 1 is absent from one or another form of living.

The second ancillary conclusion is that forms of life different from the one we know do not exist. If they do, they are based on completely different principles, and they do not fit the definition of life we refer to. Given that the composition of the Table of Elements and given that the properties of Carbon are constantly valid throughout the observed universe, other forms of life, if they exist, must be organized according to different compounds and codes. The nature of these different forms of life would have to stretch to different paradigms.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Raffaele Saladino reports financial support was provided by Italian Space Agency. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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