

# Chapter 1

## Spontaneous Generation Revisited at the Molecular Level

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**Abstract** A homogeneous chemical frame is described allowing one-pot syntheses from the one-carbon compound formamide  $\text{NH}_2\text{CHO}$  to the whole set of nucleic bases needed as precursors of nucleic acids, as we know them. Formamide also catalyzes the formation of acyclonucleosides and the phosphorylation of nucleosides to nucleotides.

The conditions are described in which the survival of these prebiotically plausible nucleotides is favored. The scenario is simple: the polymeric forms are thermodynamically favored over the monomeric ones. The consequences of this very property are relevant: the formation and the accumulation of (pre)genetic information.

### 1.1 Introduction

Asking: “where does life come from?” is the wrong way of putting the question. A possibly more correct (and empirically useful) formulation of the problem is: “how did life originate?” Not lessening the relevance of the origin of proteins and of the membrane components with all their cortege of fatty acids, the key components of the living structures as we know them are the genetic information-bearing molecules: RNA and DNA.

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Of the two categories in which the field is divided: *Metabolism-first* and *Genetics-first*, we are partisans of the latter. The reason is an Occam's Razor logics approach: without a robust system for coding and perpetuating the selection of the energy-and-matter transforming physical–chemical systems involved, the coupling of anabolic and catabolic reactions would only result in a futile series of events. In the absence of a coding apparatus, metabolic activities would not eventually lead to the ur-genetic systems capable of Darwinian selection, commonly considered to be the initial steps of evolution.

This is not meant to say that metabolism and genetics did not come to an early overlap. However, in the absence of a sufficiently firm knowledge of both categories of events, general integrative scenarios cannot at the moment be fruitfully developed further.

We have explored the possibility that a homogeneous and comprehensive chemical frame existed allowing the evolution from the simplest reactive one-carbon compound to complex informational polymers. We started focusing on one defined aspect of HCN chemistry.

## 1.2 From HCN to Nucleotides

### 1.2.1 From Formamide to Nucleic Bases

HCN is largely accepted to be the precursor molecule of nucleic bases. The major ground-breaking finding is due to Oró who reported in the 1960s the synthesis of adenosine from HCN (Oró and Kimball 1960, 1961; Oró 1961a, b). In the following decades HCN chemistry was thoroughly explored. For a detailed review see Orgel (2004), for a general overview see Delaye and Lazcano (2005). In spite of the large number of studies reporting the syntheses of nucleic bases from HCN and HCN-derivatives, the progress of the field has been limited. The low pace was essentially due to the fact that a single process (or a small number of coherent reactions) allowing the synthesis of all the basic constituents was not identified. It is all too logic to accept that in order to self-assemble an RNA molecule, all the necessary components must have been present in the same pristine test tube. Limiting for the moment the quest to nucleic bases (and according to known HCN chemistry), in the “warm little pond” first imagined by Darwin (1888) HCN alone could not have provided all the necessary building blocks. Even admitting that some of the present-day bases could have been substituted by their analogs or bioisosters (i.e., isocytosine or hypoxanthine for guanine) the HCN-alone chemistry was clearly not sufficient.

Again, an Occam's Razor logics suggested a possible solution starting from two simple observations: HCN is the most abundant three-atoms organic compound in circum- and interstellar medium and H<sub>2</sub>O is the most abundant three-atoms inorganic one (Millar 2004; [www.Astrochemistry.net](http://www.Astrochemistry.net)). The combination of HCN and

H<sub>2</sub>O affords formamide NH<sub>2</sub>COH. Reasoning in terms of probability and chemical potentialities, formamide appeared to be the obvious choice.

We have explored the capacity of formamide as a precursor of nucleic bases. In the course of several years we have analyzed the products obtained by heating formamide at temperatures between 100°C and 160°C in the presence of a comprehensive series of catalysts. The synthetic capacity of formamide was studied in the presence of:

Silica, alumina, kaolin, zeolite, CaCO<sub>3</sub> (Saladino et al. 2001)

TiO<sub>2</sub> (Saladino et al. 2003)

Montmorillonite clays (KP-10, K-30, KSF, Al-PiLC) (Saladino et al. 2004)

A set of cosmic dust analogs, from Fayalite to Olivine to Forsterite encompassing compounds with intermediate chemical composition (Saladino et al. 2005)

Phosphate minerals: Augelite Al<sub>2</sub>PO<sub>4</sub>(H<sub>2</sub>O)<sub>3</sub>, Wavellite Al<sub>3</sub>(OH)<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>(H<sub>2</sub>O)<sub>5</sub>, Hureaulite Mn<sup>2+</sup><sub>5</sub>(PO<sub>3</sub>{OH})<sub>2</sub>(PO<sub>4</sub>)<sub>2</sub>(H<sub>2</sub>O)<sub>4</sub>, Vivianite Fe<sup>2+</sup><sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>(H<sub>2</sub>O)<sub>8</sub>, Ludlamite Fe<sup>2+</sup><sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>(H<sub>2</sub>O)<sub>4</sub>, Libethenite Cu<sup>2+</sup><sub>2</sub>(PO<sub>4</sub>)(OH), Lazulite Mg[Al(PO<sub>4</sub>)(OH)]<sub>2</sub>, Vauxite Fe<sup>2+</sup>Al<sub>2</sub>(PO<sub>4</sub>)<sub>2</sub>(OH)<sub>2</sub>(H<sub>2</sub>O)<sub>6</sub>, Childrenite Mn<sup>2+</sup>[Al(PO<sub>4</sub>)(OH)<sub>2</sub>(H<sub>2</sub>O)], Turquoise Cu<sup>2+</sup>Al<sub>6</sub>(PO<sub>4</sub>)<sub>4</sub>(OH)<sub>8</sub>(H<sub>2</sub>O)<sub>4</sub>, Pyromorphite Pb<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>Cl (Saladino et al. 2006b).

Iron sulfur and iron copper minerals: Pyrrhotine (Fe<sub>(1-x)</sub>S), Pyrite (FeS<sub>2</sub>), Chalcopyrite (FeCuS<sub>2</sub>), Bornite (FeCu<sub>5</sub>S<sub>4</sub>), Tetrahedrite [(Fe,Cu,Sb)S] and Covellite (CuS) as representative of iron sulfur and iron-copper sulfur minerals differing for elemental composition (Saladino et al. 2008).

The analysis of zirconium and borate minerals is under way.

In each case a large number of nucleic bases was observed, afforded in various yields and in various qualitative and quantitative combinations. The synthesis was observed of purine, 2-aminopurine, adenine, hypoxanthine, N9-formylpurine, N9,N6-diformyl adenine, cytosine, isocytosine, hydroxypyrimidine, 2(1*H*)- and 4(3*H*)-pyrimidinone, uracil, thymine and 5-hydroxymethyl-uracil. In numerous instances compounds were also observed that are related to present-day nucleic acids biosynthetic or degradative pathways (as various imidazoles and parabanic acid) or that are known to react with aminoacids and nucleotides, as carbodiimide. The chemical rational for the various syntheses and specific quantitative aspects were reported and discussed in detail (Saladino et al. 2007; Costanzo et al. 2007a, b; and reviews quoted therein).

The general message derived from this comprehensive analysis of the formamide synthetic potentialities is that whatever catalyst is provided, a panel of different nucleic components is obtained in conditions that allow their accumulation.

### 1.2.2 From Nucleic Bases to Acyclo-Nucleosides

Of particular interest is the production of acyclonucleosides, observed as products of the photoreaction of formamide with TiO<sub>2</sub>. The formation of nucleosides by linking preformed ribose and nucleic bases is so difficult as to be considered

prohibitive in prebiotic terms (Joyce 1989; Zubay and Mui 2001). The question of how ribose and purines were linked together has been only partially solved for inosine (Fuller et al. 1972) but not for the other nucleosides. The syntheses of purine acyclonucleosides potentially alleviate the problem of the abiotic formation of nucleosides. In the presence of  $\text{TiO}_2$  formamide condenses to afford the acyclonucleosides  $N^9$ -formylpurine and  $N^9,N^6$ -diformyladenine (Saladino et al. 2003; see also Saladino et al. 2007 for a detailed description of the chemical aspects of this synthesis). The possibility that RNA might have evolved from acyclonucleosides-containing polymers has been discussed (Tohidi and Orgel 1989; Kozlov et al. 1999).

### 1.2.3 From Nucleosides to Nucleotides

Polymers will not spontaneously form in an aqueous solution from their monomers because of the standard-state Gibbs free energy change ( $\Delta G^\circ$ ), as critically reviewed (van Holde 1980). Thermodynamic considerations impose that the formation of phosphodiester linkages will be spontaneous only under highly dehydrating conditions. In the polymerization process of nucleic acids extant organisms activate the monomers by converting them to phosphorylated derivatives and then utilize the favorable free energy of phosphate hydrolysis to drive the reaction. The prebiotic alternative is that polymerization could have initially occurred in a nonaqueous environment. Thus, life did not arise in water, or (pre)genetic polymers formed from some sort of activated monomers.

Nonenzymatic synthesis of RNA chains was achieved starting from pre-activated nucleotides. The use of the phosphoramidate forms (Lohrmann 1977; Ferris and Ertem 1993; Kawamura and Ferris 1994; Kanavarioti et al. 2001; Monnard et al. 2003; Huang and Ferris 2003; Ferris et al. 2004; Mansy et al. 2008), usually phosphorimidazolides (Lohrmann 1977), shows that the accumulation of polymers is possible once suitable activated monomers are available.

Long homo- and hetero-oligomers were obtained with phosphorimidazolides using as catalyst  $\text{Pb}^{++}$  in eutectic solution (Kanavarioti et al. 2001; Monnard et al. 2003) or Montmorillonite (Ferris and Ertem 1993; Huang and Ferris 2003; Ferris et al. 2004). Earlier studies are reviewed in Orgel (2004). However, these compounds are deemed as non-prebiotic (Orgel 1998, 2004) due to the complex chemistry involved in their formation.

The concept “prebiotic” is somewhat flexible. Nevertheless, in order to be considered as such, a compound or a process should satisfy conditions of simplicity of preparation, abundance, stability and efficiency. In general terms the simpler a process, the larger is its prebiotic interest. Phosphoramidates do not satisfy this condition.

May phosphorylation of nucleosides occur in a robust and simple chemical frame? The answer is positive. In the presence of a source of phosphate and of formamide or, alternatively, of water, nucleosides are actively phosphorylated in every possible position.

### 1.2.4 Formamide

Adenosine and cytidine were analyzed as model systems of abiotic phosphorylation of purines and pyrimidines. In both instances the sugar moiety is phosphorylated—by simply heating in formamide—in the 2', or 3', or 5' positions or gives rise to the 2',3'- or 3',5'-cyclic forms (Costanzo et al. 2007a, b).

Most of the phosphorus in early Earth would have been in the form of water-insoluble minerals like Apatites. Therefore, the origin of the water-soluble (poly) phosphates required for prebiotic evolution has long been a mystery, as discussed by Schwartz (1997). It was shown that volcanic activity produces water-soluble phosphates through partial hydrolysis of  $P_4O_{10}$ , providing an at least partial solution to their origin. The phosphorylation of nucleosides to nucleotides in formamide was observed to actively occur using as phosphate donor soluble phosphate salts like  $KH_2PO_4$ , or nucleotides (i.e., 5'AMP as donor to cytidine, or 5'CMP as donor to adenosine), or one of a number of phosphate minerals. The minerals tested were: Herderite  $Ca[BePO_4F]$ , Hureaulite  $Mn^{2+}_5(PO_3(OH)_2(PO_4)_2(H_2O)_4)$ , Libethenite  $Cu^{2+}_2(PO_4)(OH)$ , Pyromorphite  $Pb_5(PO_4)_3Cl$ , Turquoise  $Cu^{2+}Al_6(PO_4)_4(OH)_8(H_2O)_4$ , Fluorapatite  $Ca_5(PO_4)_3F$ , Hydroxylapatite  $Ca_5(PO_4)_3OH$ , Vivianite  $Fe^{2+}_3(PO_4)_2(H_2O)_8$ , Cornetite  $Cu^{2+}_3(PO_4)(OH)_3$ , Pseudomalachite  $Cu^{2+}_5(PO_4)_2(OH)_4$ , Reichenbachite  $Cu^{2+}_5(PO_4)_2(OH)_4$ , Ludjibaite  $Cu^{2+}_5(PO_4)_2(OH)_4$ . The results are detailed in Costanzo et al. (2007a, b). The largely more active minerals were found to be Reichenbachite, Ludjibaite, Libethenite and Cornetite, all of which are copper phosphates. Thus, a simple and robust phosphorylation process could have been possible in the same chemical frame and in the same physical–chemical conditions (formamide and heat) in which nucleic bases could have formed and evolved to their nucleoside forms.

### 1.2.5 Water

Nucleoside phosphorylation also occurs in water, in conditions similar to those described (Costanzo et al. 2007a, b) for formamide. In water phosphorylation is slower than in formamide (the plateau level being reached in water, in the adenosine model system analyzed, after 100 h relative to the 25 h needed for formamide). The fraction of the phosphorylated nucleoside molecules is also lower (7% relative to 25% in formamide) and only occurs in the presence of high concentrations of phosphate donor molecules (1M  $KH_2PO_4$  was observed to be most the efficient concentration). The degradation of nucleotides back to nucleosides and nucleic bases is rapid, the half lives of the various phosphorylated forms being encompassed between 20 and 50 h. Nevertheless, these results show that phosphorylation occurs in a simple solvent (water), in acceptable temperature conditions (90°C) and at moderate and non-fastidious pH values (between 4.0 and 8.0).

This set of observations (Costanzo et al. 2008) points to the prebiotic possibility of obtaining activated forms of nucleosides by simply treating them in the presence



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