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# Origins of Life: Molecular Foundations and New Approaches

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*Dedicated to the Memory of Sir* ***Derek Barton***

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## 1. Introduction

The emergence of life on Earth is the result of a long chemical evolution which began ca. 4.5 thousand million years ago. The oldest organisms, of which fossilized remains have been found in carbon-rich rocks, were living in an environment devoid of dioxygen *ca.* 3.8 thousand million years ago. How is it possible to explain the prebiotic synthesis of biomolecules ? What laboratory experiments can be carried out to explain in molecular terms the appearance of a system capable of synthesising the first metabolic links and of transmitting genetic information ? We present here experimental approaches and recent results that have allowed the formulation of plausible scenarios.

## 2. The Origin of Organic Molecules on Earth

### 2.1 The primitive Earth conditions

It is estimated that the Earth formed 4.5 thousand million years ago by accretion of material from the solar nebula, through processes which may have lasted hundreds of millions of years.[1,2] The oldest known sedimentary rocks containing carbon molecules of biological origin have been found in the West of Greenland and date from 3.8 thousand million years.[3,4] The conditions which existed at this time on the surface of the planet were totally different from those we know today.

As regards the composition of the primitive atmosphere, different models based on astrophysical, geological and geochemical data have been proposed.[5] The most famous amongst them, controversial today, proposed a composition rich in reduced gases like methane, ammonia and water vapour.[6,7] In fact, the analyses of trapped gases in ancient rocks and the existence of sedimentary carbonate deposits dating from this time suggest that the primitive atmosphere was much less reducing.[8] It is now thought that an atmosphere, known as secondary, would have been formed in the course of the cooling of the planet as a result of volcanism and the progressive degassing of the crust and mantle. The volcanoes must have expelled huge amounts of gas which contributed to the

formation of an atmosphere containing carbon dioxide, water vapour, sulfur dioxide, maybe small quantities of carbon monoxide, methane and dinitrogen, but no dioxygen.[9] When the temperature of the crust fell below a critical level, large volumes of liquid water accumulated by condensation and formed the oceans. Moreover, 4 thousand million years ago the sun only emitted 75% of the energy it does today. This energy deficit would normally have entailed a glaciation of the Earth if a compensating greenhouse effect linked to the considerable amounts of carbon dioxide had not allowed the maintenance of water in its liquid state. The heat given off from an intense radioactive decay, particularly that of potassium-40 at this time, no doubt contributed to the maintenance of the relatively elevated temperature.

These are, very briefly, the extreme conditions in which life on Earth had to take its first steps. Today's life forms, such as we know them, are by consequence the result of an evolution process of approximately 4 thousand million years duration.

Paleontological discoveries in Precambrian soils show that for the first 3 thousand million years the only living systems on our planet were micro-organisms.[4,10] The simplest Precambrian micro-fossils show striking similarities with present day algae and cyanobacteria. Numerous microfossils have been discovered in the silicified parts of fossil stromatoliths, lamellar structures formed of cellular alignments, very similar to those formed today by certain bacteria and cyanobacteria.

Today, there is a general agreement that life was already present on the primitive Earth around 800 million years after its formation.[4,11]

### 2.2 Prebiotic Chemistry

What happened during these 800 million years of the Earth's history, which led to the appearance of organisms similar to present day bacteria ? Can we reconstitute in the laboratory, under the supposed primitive conditions, the early stages of bioorganic molecular synthesis ?

Chemical evolution led the simplest elements, hydrogen, carbon, oxygen, sulphur, phosphorus etc... to combine to form the organic molecules methane, carbon

monoxide, carbon dioxide and water vapour, molecules which, as we have seen, were present in the atmosphere of the primitive Earth. If a gaseous mixture of these molecules is heated to a high temperature and quenched, or submitted to an electrical discharge, structurally complex organic molecules are formed.[12-14]

Such syntheses, carried out in the laboratory from simple molecules under conditions believed to represent initial terrestrial conditions, constitute what is called prebiotic chemistry. Strictly speaking, one should however distinguish between "abiotic" chemistry, which can lead to the synthesis of substances present in extant living organisms, but not necessarily under conditions which could have led to the transition to living organisms, and "prebiotic" chemistry, possibly relevant to the emergence of life.

For instance, the first abiotic synthesis of a natural product was that carried out by the German chemist Friedrich Wöhler in 1828,[15] when he obtained urea from an inorganic substance, silver cyanate (*via* cationic exchange to give ammonium cyanate then thermal decomposition). This showed for the first time that it was possible to cross the barrier which separates mineral chemistry from organic, living, chemistry.

However, the date 1828 is not usually mentioned in the history of the molecular origin of life, as the work of Wöhler was not linked to this problem, and the conditions used are definitely not "prebiotic". Moreover, Wöhler's work predates Darwin's publication on *The Origin of Species* (1859), and only the most advanced spirits of the time would allow themselves to consider that Life could have evolved from inert matter.

One of the most important prebiotic reactions, and one of the simplest to carry out, is the formation of hydrogen cyanide from dinitrogen and methane.[16,17] Another possible prebiotic synthesis, from simple compounds in a gaseous state, is the formation of formaldehyde from methane and water vapour. Hydrogen cyanide and formaldehyde are probably amongst the atmospheric precursors which, dissolved in the water of the oceans, lagoons or lakes, could have reacted spontaneously to give more complex compounds, the elementary building blocks of life.

### 2.3 The Prebiotic "Soup"

Oparin and Haldane, in 1924 and 1929 respectively, proposed that hydrogen cyanide and formaldehyde could have formed in water a primitive soup where complex molecules would have formed spontaneously, and would have at an appropriate time given rise to life as we know it today. The protocells or coacervates initially formed would have drawn from the primitive broth the molecules capable of putting in place a first heterotrophic metabolism.[6]

Part of this hypothesis has been tested experimentally. In 1953, Miller, working in the laboratory of the chemist Urey, submitted a gaseous mixture consisting of water, ammonia, methane and dihydrogen, the then assumed compostions of primitive atmosphere, to an electrical discharge.[12,13] The results were striking : after one week, Miller found that more than half of the 20 amino acids found in living cells today were formed in significant quantities. They were obtained after the initial formation of hydrogen cyanide and formaldehyde.

While the conditions selected by Miller for his atmosphere were later criticized, his experiment led to further important results. In the 1960s, Oró succeeded in synthesising the purine base adenines[18,19] and guanine[20] from aqueous ammonium cyanide (Figure 1). Adenine was also obtained photochemically from hydrogen cyanide (Figure 1).[21,22]

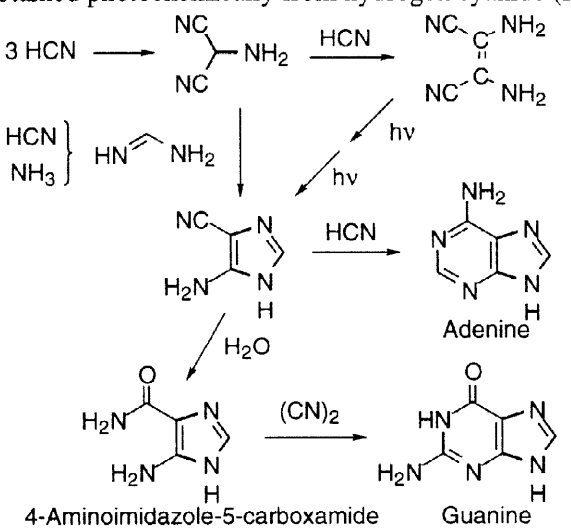



Figure 1: Synthesis of the purine bases adenine and guanine from hydrogen cyanide.[18-22]

It is not possible to summarise in only a few words the very considerable amount of work carried out in the last forty years (see[23,24] for reviews). The ease with which

these reactions occur is a strong argument to make them very good candidates as the source of biochemical monomers.

However, this scenario of the prebiotic soup has a certain number of weaknesses. The first concerns the low concentration of the precursors ; the high dilution of organic compounds in the wide aqueous environment of the oceans would be an obstacle to their reacting and hence to the synthesis of more complex compounds. A second problem, linked to the presence of water as a solvent, would be the hydrolysis of the reactants and of the reaction products. A third problem would be that of selection: in a primitive broth one would have as much chance a priori of obtaining toxic substances, anti-metabolites, as molecules useful for the purpose. Numerous reactions could interfere with the favorable processes.

### 2.4 Interstellar Organic Chemistry

Other sources of organic molecules have been considered. Recent assessments indicate that very significant quantities of organic material from meteoritic and cometary sources have been deposited on the primitive Earth (approximately 20 g/cm$^2$).[25-28] It seems that organic synthesis is very active in the interstellar space. For example, hydrogen cyanide, adenine and polymers of formaldehyde have been identified in comet Halley. Seventeen amino acids, in every way identical to those made in the course of the Miller experiment, have been identified in the Murchison meteorite which fell on Australia in 1969.[5,27]

It is estimated that more than 100 tonnes of meteorites now fall on our planet in one year, and that, at the time of formation of the Earth, the bombardment was 10 000 times more intense; thus, meteorites have brought an enormous quantity of organic molecules to the Earth.[29]

### 2.5 Hydrothermal Sources and Sulfur Chemistry ; the "Thioester World"

Finally, it is also possible that part of the compounds necessary for life were produced in the high temperature vents present in the deep oceanic ridges. In the 1980s, investigations from the "Nautilus" deep-sea research vessel led to the completely unexpected discovery of hot springs at depths greater than 2600 metres. These marine vents resemble small volcanic cones, the sides of which are covered with fissures through which sea water can infiltrate and come into contact with the hot basalt. When this water emerges, its temperature can be as high as 400°C, it is more acidic and it is enriched in mineral salts and in metallic elements. These marine vents which continuously expel large quantities of hydrogen sulphide and derivatives such as dark metallic sulfides (hence their name "black smokers"), constitute a reducing environment containing all the necessary ingredients for prebiotic chemistry. Today, living communities constituting genuine independent food chains have developed around these black smokers without using solar energy.

What are the arguments to assume that these hydrothermal springs may have been the place of the origin of life ? Corliss has called attention to the discovery, in geological sediments dating from 3.5 thousand million years, of micro-fossils resembling forms now found in hydrothermal zones.[30] This new area of research probably holds many more surprises.

In 1997, Huber and Wächtershäuser have demonstrated the potential chemical relevance of the "black smoker" environment in prebiotic synthesis.[31] They have shown that mixed iron nickel sulfides act as a catalyst for the conversion of methyl thiol and carbon monoxide, present in vent gases, to methyl thioacetate, a compound containing an activated acetyl group such as that in acetyl-coenzyme A, a central intermediate in the biosynthesis of important biomolecules (Figure 2). This biomimetic C–C bond-forming reaction can therefore be considered as a preliminary step in the building up of more complex organic molecules in or near the early oceanic vents.[32]

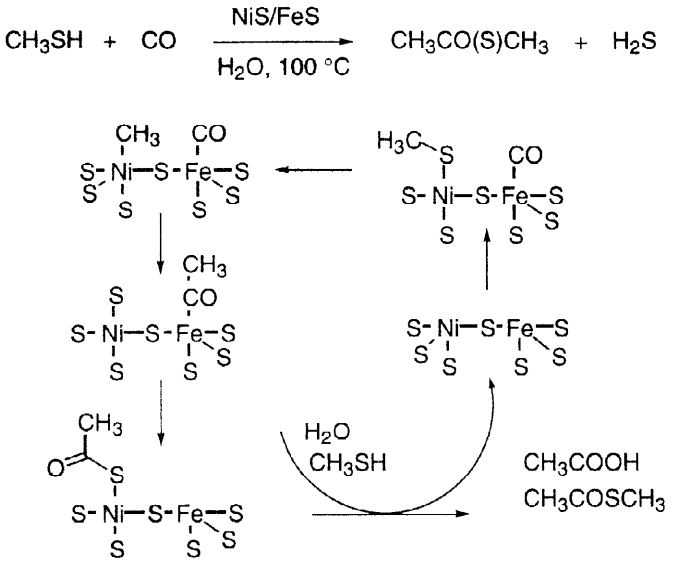



Figure 2. The first evidence of a possible prebiotic chemistry in the "black smoker" environment : C–C bond-forming conversion of methyl thiol and carbon monoxide into methyl thioacetate catalysed by co-precipitated nickel (II) and iron (II) sulfides and representation of a hypothetical mechanism.[31]

Sulfur chemistry may have been essential to the prebiotic synthesis of bioorganic molecules. Some microorganisms are able to use thioester intermediates for the synthesis of small proteins in the absence of the highly ordered apparatus provided by the ribosome and tRNA structures. Lipmann[33] and de Duve[34,35] proposed that a thioester-dependent mechanism of peptide bond formation may have preceded the RNA-dependent mechanism of protein synthesis in the earliest steps of life. Experimental evidence of peptide bond formation by self-condensation of aminoacyl thioesters has been reported.[36-39] In the "Thioester World" hypothesis of de Duve (more precisely a thioester-iron world), thioesters would also be reagents for phosphorylation and a primary energy source for the early biogenic processes. In 1997, Liu and Orgel described a closely related route to amide formation involving acylation by thioacetic acid and an oxidizing agent ; this is of interest as it would provide a route to prebiotic peptide synthesis and ligation in an aqueous solution.[40]

Hartman from 1975,[41] then Wächtershäuser from 1988[42] proposed a primordial autotrophic metabolism. Wächtershäuser suggests the possibility of a surface-based "organism", in which simple negatively charged organic molecules become fixed on the positively charged pyrite surface and are able to use atmospheric carbon dioxide directly

as a carbon source, exactly as is done by green plants and certain bacteria today. Such an organism would have developed a "surface metabolism" at the time of the origin of life on Earth. It can be mentioned here that iron-sulfur redox proteins containing a cage-like cluster of iron and sulfur linked to cysteine residues of the peptide chain are widely distributed in Nature (for example rubredoxins, adrenodoxins, ferredoxins). They are involved in electron transfers and associated or implicated in a large number of metabolic reactions especially in photosynthetic processes and $CO_2$ and $N_2$ fixation in bacteria and plants. Wächtershäuser's theory has been extensively developed, and constitutes, despite the paucity of experimental results, a major novel approach.

### 2.6 The Origin of Bioorganic Homochirality

The origin of chirality in living molecules (e.g. the exclusive involvement of L-amino acids and D-ribose) is at present not satisfactorily explained (even though it would be very difficult to conceive of a "racemic world"); we can at the moment only hypothesise, for example that natural chiral minerals may have played a role, and/or have induced a selection by the specificity of certain reactions.[43,44] In 1997, Engel and Macko have demonstrated that the Murchison meteorite contains an enantiomeric excess of certain L-amino acids (for alanine and glutamic acid, over 30 and 50%, respectively) that are, today, almost exclusively present in living systems.[45] These results suggest that an extraterrestrial source for an excess of L-enantiomers in the Solar System may predate the origin of Life on Earth.[45,46] This excess may have resulted from the alteration of initially abiotic racemic mixtures by a process such as preferential decomposition by exposure to circularly polarized light.[43,47]

To conclude this brief overview, it can be seen that there are several plausible hypotheses which can account for the presence of the elementary building blocks of life on the primitive Earth.

## 3. Selection of an Essential Function: Self-Replication

In an examination of cellular function, how can we trace the key steps in the development of the early Life ?

### 3.1 What Are the Respective Roles of Nucleic Acids and of Proteins in the Genesis of Life ?

Proteins play many functions in a cell. They play a structural role and are associated with other molecules (in particular phospholipids - see Addendum) to form the cell membrane. They also intervene in the control and in the transport of molecules to the interior of the cell and in the transmission of information (in the form of molecular signals) between cells. Many of them are enzymes, the bio-catalysts of chemical reactions in the living world. Proteins are made from a range of at least 20 amino acids, each of which possesses distinct chemical properties. The chains formed by amide bonds are strictly organized in helices, pleated sheets, and various types of domains.

The nucleic acids DNA and RNA (Figure 3) play the cellular role of storage and transfer of information. The genetic information held in the form of genes, contained in the DNA of the mother cell, must be conserved in an exactly (or nearly exactly: changes are required for evolution) replicated form in the descendants.

The DNA molecule is made up of the association of two strands wound in a helix but the RNA molecule is generally a single strand. Several arguments lead to the idea that RNA predated DNA in evolution.[48-51] Among these, it is known that the living cell makes deoxynucleotides by reduction of ribonucleotides.[24,52] Thymine, a base specific for DNA, is obtained by the transformation of uracil, which is specific for RNA (Figure 3). Furthermore, RNAs are required primers in the synthesis of DNA, whereas the synthesis of RNA is carried out without recourse to DNA. It can therefore be considered that DNA is an RNA which has been modified in order to fit it for efficient storage of genetic information.

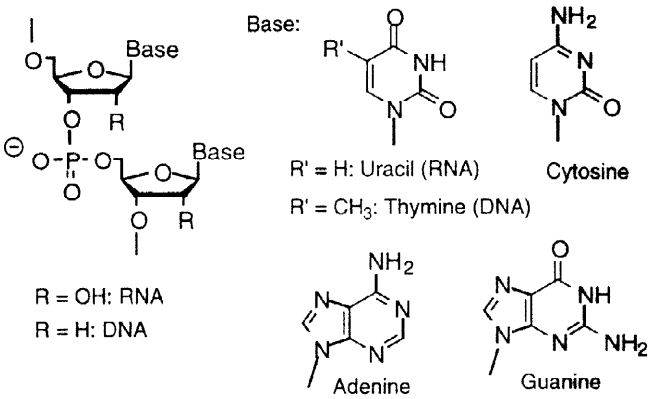



Figure 3. Structures of RNA and DNA

In the "RNA World" hypothesis of the origin of life,[53,54] RNAs are assumed to be the central macromolecules able to self-replicate by base pairing, conserve information, and catalyse the reactions necessary for a primitive metabolism.

Many enzymatic cofactors possessing in their structure a ribonucleotide moiety may be regarded as molecular fossils of the "RNA world" (see section "Minor bases and fossil cofactors"). The discovery of catalysis by RNAs called ribozymes is another argument for the primacy of RNAs in evolution (see section "RNA and catalysis"). Nature may have solved the problems associated with catalysis and substrate recognition by generation and screening of large populations of RNA molecules. Recently, methods have been developed for generating and screening large libraries of nucleic acid molecules.[55-60] This *in vitro* selection led to new RNAs, called aptamers, able to bind selectively and strongly various biomolecules, for example amino acids, ATP, flavin and nicotimamide redox cofactors ($K_d$: $10^{-3}$-$10^{-9}$ M). New ribozymes able to catalyse various reactions were also selected (see section "RNA and catalysis"). Structural studies have revealed that these RNAs can fold to form selective binding pockets.[59,61,62]

### 3.2 Self-Replication and Experimental Models

Ancestral RNAs must have been able to assemble themselves from a soup of precursors and to evolve in self-replicating patterns, using recombination and mutation to explore new functions.[53] If we are beginning to understand the replication of nucleic acids in extant organisms, the same cannot be said for the origins of the replication mechanism. When they replicate, the two nucleic acid strands separate, and by

complementarity each one of them serves to regenerate the missing strand. In the cell, there are very specialised enzymes which carry out this task ; under primitive conditions, we have to suppose that replication may have occurred without the intervention of enzymes, by the simplest possible template-directed synthesis.

• *Template-directed synthesis*

The principle of this system, studied since the early 1970s by Orgel, is now well known.[22,63-65] Mononucleotides activated in the form of 5'-phosphoro(2-methyl)imidazolides (model coupling reaction ; Figure 4) are positioned according to the pairing rules of Watson and Crick on the surface of a preformed polypyrimidine template and, because they are activated, they are able to link to one another to form the complementary strand (Figure 4). In this way, many defined oligonucleotide sequences are copied faithfully. However, it was not possible to find a pair of complementary sequences, each of which facilitates the synthesis of the other.[65] The chief obstacles are the formation of intramolecular and intermolecular complexes (for example tetraplexes). Moreover, the template-directed polymerisation of one enantiomer is likely to be strongly inhibited by the presence of the other (enantiomeric cross-inhibition).[66] The template-directed synthesis of RNA could have been more efficient and selective by assembling short activated oligonucleotides.

Other completely artificial systems of replication have been investigated.[67-70]

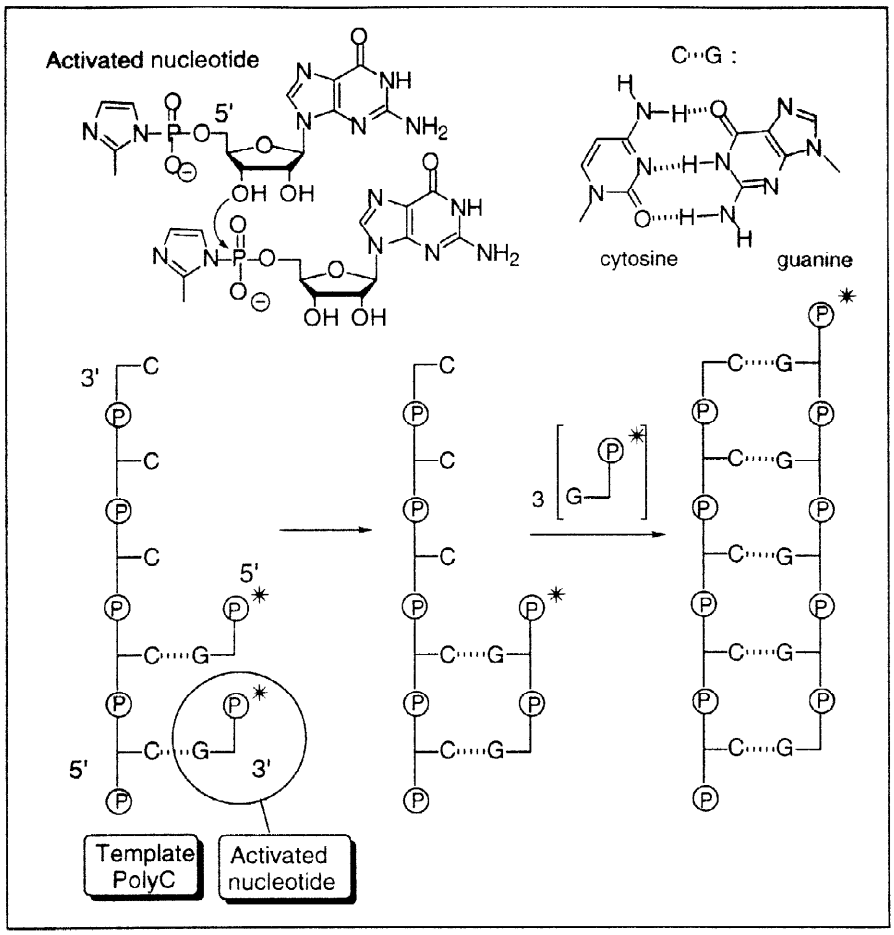



Figure 4. Template-directed synthesis of oligonucleotides.[22,63-65]

• *The problem of the original template and of D-ribose*

Several difficulties were encountered in the synthesis of all the pieces of an RNA molecule under the primitive conditions.[63,71] The work of Oró[18-20] showed that, under potentially prebiotic conditions, purines could easily be obtained but pyrimidines only with difficulty.[72] When one tries to produce ribose from formaldehyde and glycolaldehyde in the presence of sodium hydroxide and calcium acetate, by the so-called "formose reaction",[73,74] a very complex mixture is obtained, in which ribose is only a very minor component. Moreover, ribose is unstable on a geological time scale.[75] However, pentose and hexose phosphates can be obtained in good yields from glycolaldehyde phosphate, especially ribose 2,4-diphosphate predominating over hexose

triphosphates when the reaction is run in the presence of formaldehyde (see next section).[76]

In the following step of the RNA synthesis, the nucleoside assembly, additional problems of selectivity are encountered. Heating of a mixture of purine base and D-ribose gives a mixture of isomers in which the configuration of the sugar, the location of the base and the relative orientation of the two rings are mostly different from those of the β-D-ribonucleoside of our cells.[77,78]

### 3.3 RNA Simplified or Modified

In theory, the ribose-phosphate backbone (Figure 3) may not be required for the transfer of genetic information and a simpler system of replication appearing before RNA can be envisioned. Furthermore, it is postulated that such a pre-RNA molecule needed to be more robust than the sensitive and complex components of the RNA World. Work has been directed towards the synthesis and study of molecules which can self-replicate more simply than contemporary RNA by replacing the ribose by acyclic compounds such as glycerol, acrolein or erythritol.[71,79,80] Danish chemists replaced the ribophosphate backbone by amide links similar to those in proteins (Figure 5).[81-87] These new nucleic acid analogues called PNA (for peptide nucleic acids) are capable of strongly pairing with oligodeoxyribonucleotides according to the rules of Watson and Crick. PNA can act as templates that can catalyse the non-enzymatic synthesis of their RNA complements from activated mononucleotides and *vice versa*.[86] This suggests that a transition between different genetic systems would be possible without loss of information.

Eschenmoser studied an alternative structure, an isomer of RNA, p-RNA, in which the sugar is in the pyranose form (Figure 6).[23,76,88-92] This proposal came from studies in the aldomerization of glycolaldehyde phosphate which could be obtained under presumed prebiotic conditions. In the presence of formaldehyde, a ribopyranose derivative, racemic ribose-2,4-diphosphate, was obtained as the kinetically favored product (Figure 6).[76,91-92] An experimental approach was developed to answer the

questions "why pentose- and not hexose-nucleic acids ?" and "why did Nature choose furanosyl-RNA and not pyranosyl-RNA as its molecular genetic system". After considerable work on the synthesis and studies of different pentose and hexose RNA and DNA analogs, (4' → 2')-ribopyranosyl-RNA (Figure 6) was found to have a stronger and a more selective pairing system than natural RNA. In 1997, Bolli *et al.* demonstrated that base-sequences of p-RNA can be copied by template-controlled replicative ligation of short activated oligomers under mild conditions and that this ligation is highly chiroselective.[44]

In order to overcome the problem of enantiomeric cross-inhibition encountered in the oligomerization of ribonucleotides,[66] Sutherland recently proposed achiral monomers which could aldomerise to form an RNA precursor (Figure 7).[24] In this interesting approach, a route involving a retro-Amadori rearrangement was suggested to obtain RNA from this pre-RNA. The Amadori rearrangement involves aza-enolisation followed by ketonisation and is known in the contemporary biosynthesis of tryptophan, histidine and several coenzymes. Monomers containing each of the four RNA bases were synthesised and preliminary results of polymerisation of the adenine and uracil derivatives were reported.[93] At room temperature in water at pH 9.5, short oligomers were formed. The exact nature of the oligomeric products remains to be established but it was shown that the oligomerisation involves aldol reactions.

Aldol reactions may have been implicated in the synthesis of RNA precursors other than ribose. In this regard, we are studying condensations between nucleic bases and different aldehydes under presumed prebiotic conditions to obtain nucleoside-like building blocks.[94]

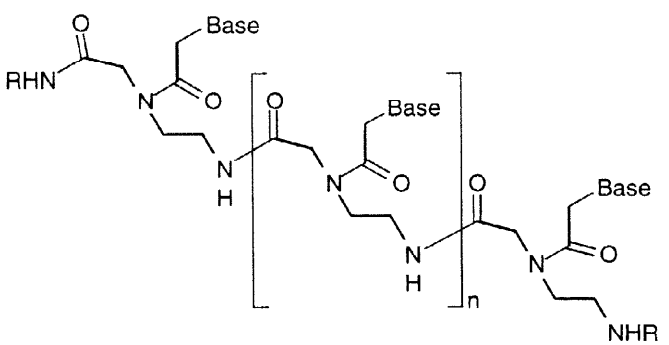



Figure 5. Structure of peptide nucleic acids (PNAs).[81-86]

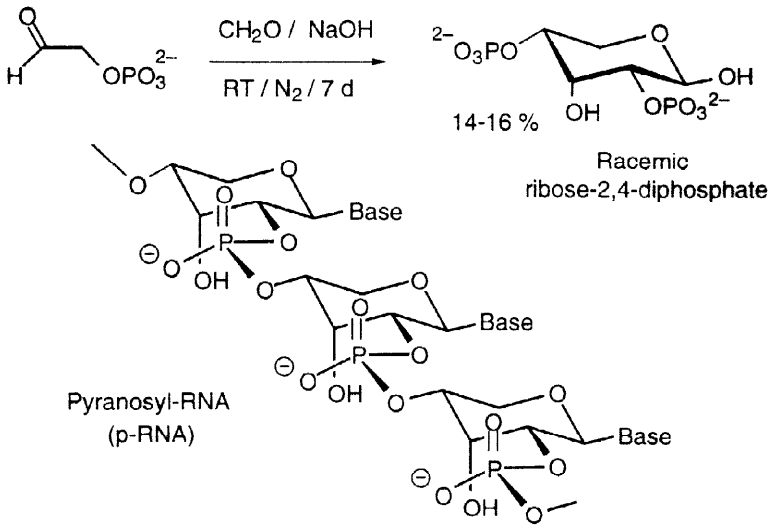



Figure 6. Aldomerization of glycolaldehyde phosphate in the presence of formaldehyde and constitution and configuration of the (4'-2')-ribopyranosyl isomer of RNA (p-RNA).[23,44,76,88-92]

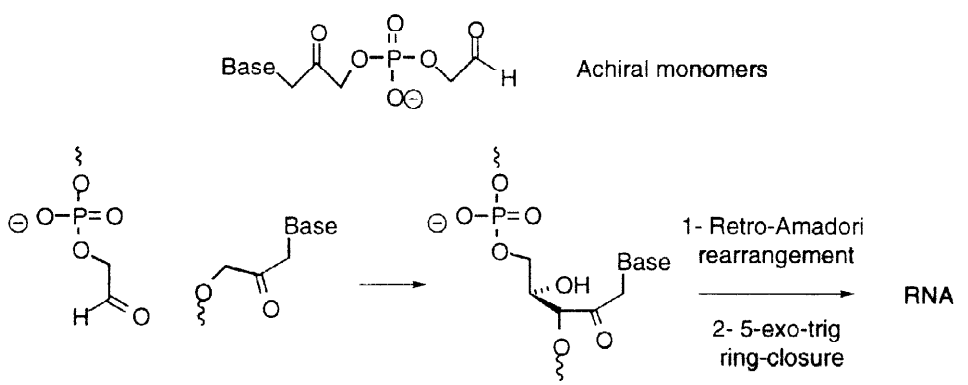



Figure 7. Achiral monomers and corresponding polymer proposed as RNA precursors by Sutherland.[24,93]

## 4. Primitive Catalysis

### 4.1 Homogeneous or Heterogeneous Catalysis ?

In the field of primitive catalysis, work has been pursued on several fronts. Some authors proposed that certain clays or small peptides can play the role of catalysts.[95-99] The British crystallographer Bernal suggested in 1951 that the absorption of molecules onto a mineral surface, for example clay, could facilitate their polymerisation.[100] These minerals have a laminar structure characterized by alternate sheets a few Å thick,

positively or negatively charged. Amino acids can be fixed between the layers of clay in such a way as to facilitate their condensation.

In the 1970s, Paecht-Horowitz and Katchalsky carried out an experimental demonstration of this theory.[95,96] They showed that a particular clay, montmorillonite, acts like a mini-reactor : it stores, concentrates and positions the adenylated amino acids between its layers and favours their polymerisation. From this point of view, the clay can be considered as a primitive enzyme. Similarly, several researchers succeeded in condensing mononucleotides or amino acids on the clay surface.[101-103]

The idea of a role for mineral surfaces in the origin of life has gathered a following and it has been developed parallel to work linked to the hypothesis of the prebiotic soup.

### 4.2 RNA and Catalysis: Ribozymes

For a long time, it was thought that there was an absolute separation in the living cell between the roles of nucleic acids as information carriers on the one hand, and of proteins as catalysts on the other. In the 1980s, Altman and Cech showed that certain ribonucleic acids (ribozymes) have catalytic functions.[104-106]

The idea that RNAs might have catalytic activity had been proposed more than 30 years ago.[49] This hypothesis was put forward for the first time by Woese in 1967,[107] then reiterated in 1968 by Crick[108] and Orgel[48]; in 1979, Weber and Orgel even demonstrated the role of polyurydilic acid in the course of the synthesis of a small glycine-glycine peptide.[109]

Today it is recognized that ribozymes can display several properties.[60] The first is their ability to carry out self-splicing reactions, that is to say the cutting and joining end-to-end of fragments of an RNA molecule, that proceeds without proteins, by two transesterification reactions (Figures 8 and 9).[110] It is also the case in the self-hydrolysis of several viroids or when one RNA molecule acts on another, for example RNase P, an enzyme important in transfer RNA maturation.[111] Large ribozymes require divalent metal ions such as magnesium ions to fold correctly.[112-113] Divalent metal ions, such as

magnesium, manganese, cobalt, calcium cations, are also essential for efficient catalysis by ribozymes (Figure 10).[114-116]

Figure 8. Transesterification involved in the hydrolysis of RNA or its splicing catalysed by ribozymes.

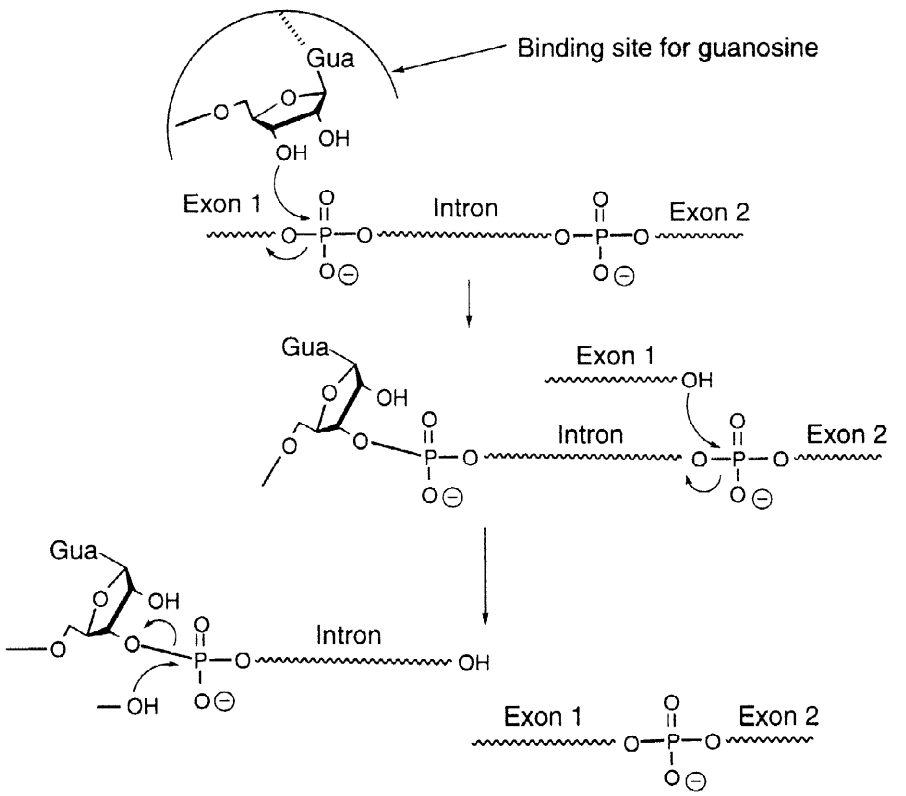



Figure 9. Splicing of RNA catalysed by ribozymes.

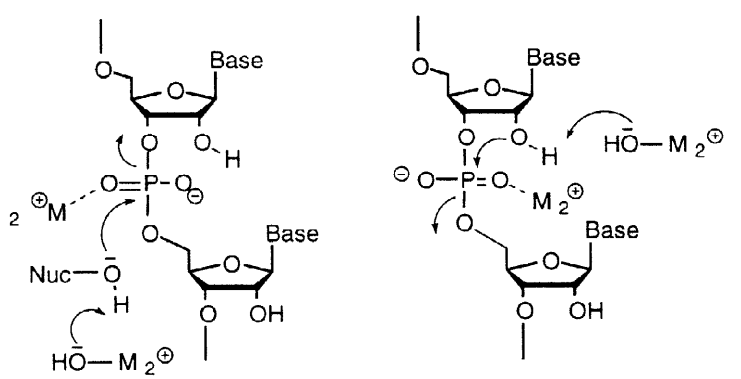



Figure 10. The two mechanistic classes of ribozyme phosphodiester cleavage. $M^{2+}$ represent divalent cations.[114]

In these examples, the RNA molecule acts on itself. In 1992, Noller and his collaborators showed the possible catalytic role of 23S ribosomal RNA during protein synthesis.[117] In 1997, Zhang and Cech demonstrated that ribozymes obtained by *in vitro* selection perform the same peptidyl transferase reaction as the ribosome for linking aminoacids. [118] It may be that the peptidyl transferase of our ribosomes comprises only RNA.[119-121]

Ribozymes or 2'-deoxyribozymes (DNA) possessing new catalytic properties were obtained by *in vitro* selection of nucleic acids or by modification of natural ribozymes and/or their substrates.[55-60]

Selected ribozymes are able to catalyse the synthesis of complementary strand RNA,[122] DNA cleavage[123,124] or amide bond cleavage[125]. A shortened version of a *Tetrahymena thermophila* ribozyme was shown to catalyse the self-incorporation of coenzyme analogs such as $NAD^+$ and dephosphorylated CoA-SH.[126] Similar ribozyme activities may have played an important role in the "RNA World" by expanding the limited number of chemical functional groups necessary for maintaining a complex metabolism in the absence of proteins (see section "Minor bases and fossil cofactors").

Ribozymes able to perform new reactions were selected for their ability to bind strongly transition state analogs, for instance ribozymes that catalyse isomerization of one of the diastereomers of a biphenyl derivative[127,128] (Figure 11) or porphyrin metalation[129] (Figure 12). In 1997, pyridine-modified RNA molecules that catalyse a Diels-Alder cycloaddition were successfully selected (Figure 13);[130] this was the first example of a carbon-carbon bond formation catalysed by a ribozyme.

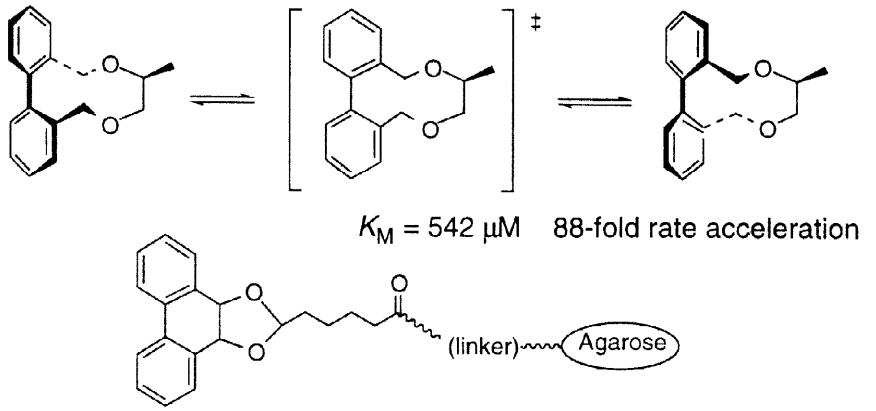



Figure 11. Isomerisation of a diastereomeric biphenyl derivative catalysed by a 165 base pairs RNA that was selected for its affinity for the near-planar transition state analog coupled to an amino-derivatised cross-linked agarose support.[127-128]

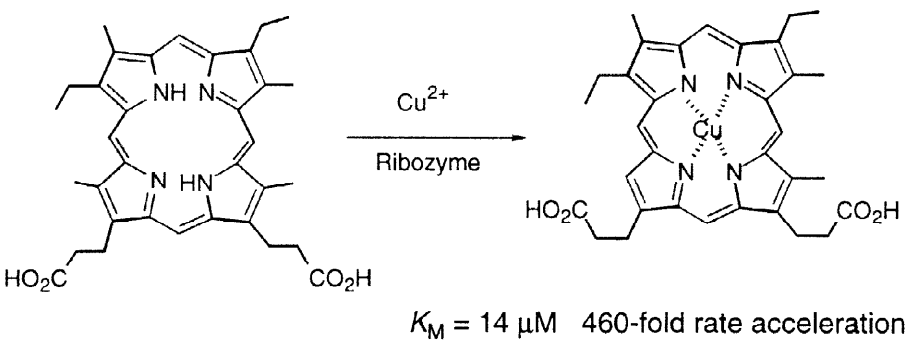



Figure 12. Metalation of mesoporphyrin catalysed by RNA molecules selected for their affinity for *N*-methylmesoporphyrin in both the presence and absence of $Cu(OAc)_2$.[129]

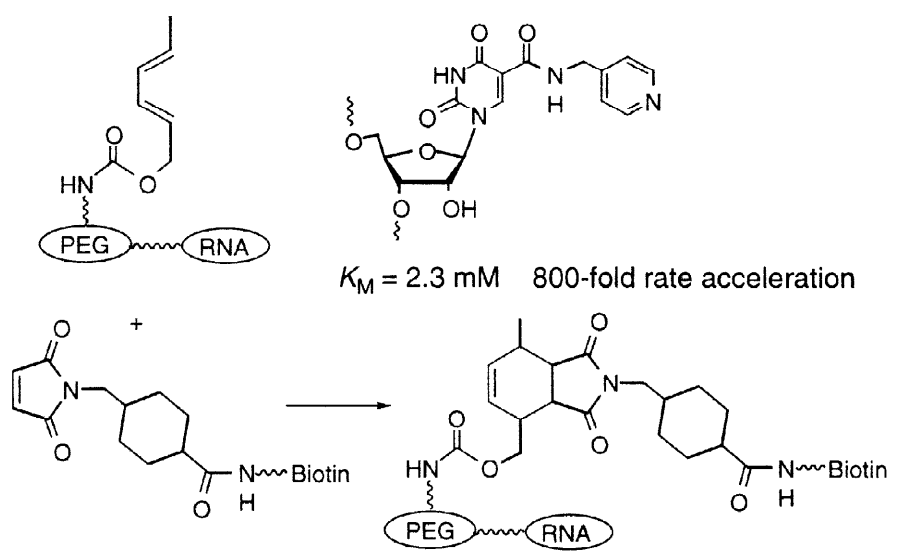



Figure 13. The Diels-Alder reaction between the acyclic diene conjugated to a 100 base pair RNA through a long PEG linker and the maleimide dienophile attached to biotin for RNA selection using streptavidin binding. The RNA was modified by substituting 5-pyridylmethylcarboxamid-UTP C[13] for UTP in the transcription reaction. Within the ribozymes, the modified base could form pyridyl-Lewis complexes with transition metal ions. The presence of Cu (II) ions is absolutely required for catalysis.[130]

The existence of these RNA catalysts poses questions concerning primitive catalysts relevant to the origin of life :[131] were the first molecules related to our nucleic acids also endowed with catalytic activity ? In this context, it is interesting to explore catalysis by nucleic acid-like molecules. The necessity of incorporating a modified base in order to obtain a ribozyme which catalyses a Diels-Alder cycloaddition demonstrates the interest of such an approach (Figure 13).[130]

### 4.3 Minor bases and fossil cofactors

RNAs can acquire the majority of functional groups possessed by the amino acids in proteins by chemical modification. When the functional groups do not exist in the initial monomers, the macromolecules can acquire them by post-transcriptional modification or by cofactor addition.

Transfer RNAs participate in protein synthesis by positioning the amino acids in order for them to react according to a programmed linking. These RNAs have a particular structure: after post-transcriptional modification they carry a large number of modified bases, called minor or rare bases, the role of which is poorly understood (79 such bases were listed in 1994).[132,133] A large number of different chemical functions can be found on these bases, including even amino acids. These bases might have filled the catalytic role in one of the stages of evolution in the same way as cofactors do in modern enzymes.

Certain enzymes function with the aid of small exogenous molecules derived from vitamins. These cofactors are natural reactants distinctive because their presence is required for enzymatic function. A large number of them are derived from a ribonucleotide, adenosine 5'-phosphate, which reinforces still further the idea of the antecedence of an RNA with catalytic properties.[134-136]

On the other hand, certain structural elements of these cofactors can be obtained under postulated prebiotic conditions. For this reason, these compounds are often considered as "molecular fossils" implicated in the first reactions which produced life. Such is the case of riboflavin,[23,137,138] nicotinamides ($NAD^+$, $NADP^+$),[139-141] vitamin B12 or cyanocobalamine.[142] For example, riboflavin, normally synthesised in a series of enzymatic reactions, can also be obtained under extremely simple conditions (see

references above; Figure 14). Recently, pantetheine, a precursor of coenzyme A, which is an acyl group carrier, has been prepared under conditions which can be considered as prebiotic (Figure 15).[143,144]

RNA aptamers which strongly bind some of these cofactors were successfully selected, for example cyanocobalamine ($K_d$ = 88 nM)[145] and flavin and nicotinamide cofactors (0.5-100 μM).[146,147]

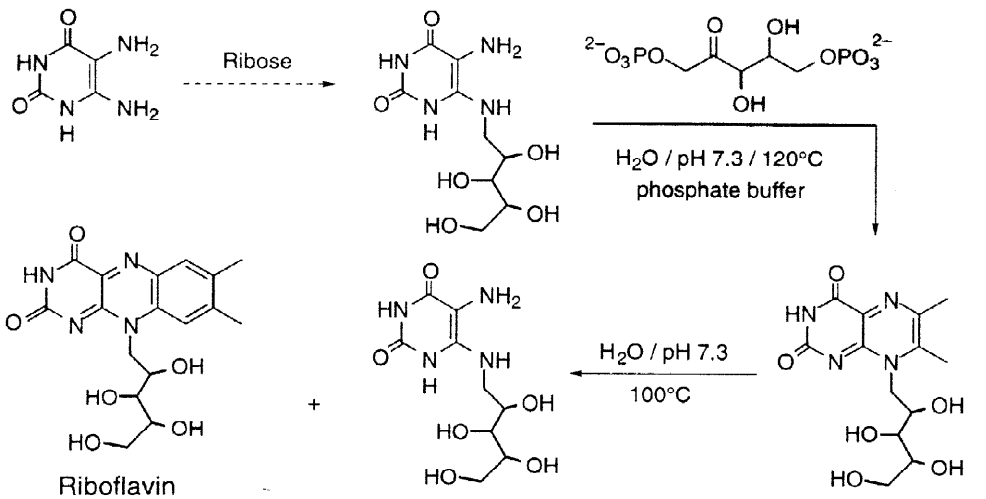



Figure 14. A possible prebiotic synthesis of riboflavin.[23,137,138]

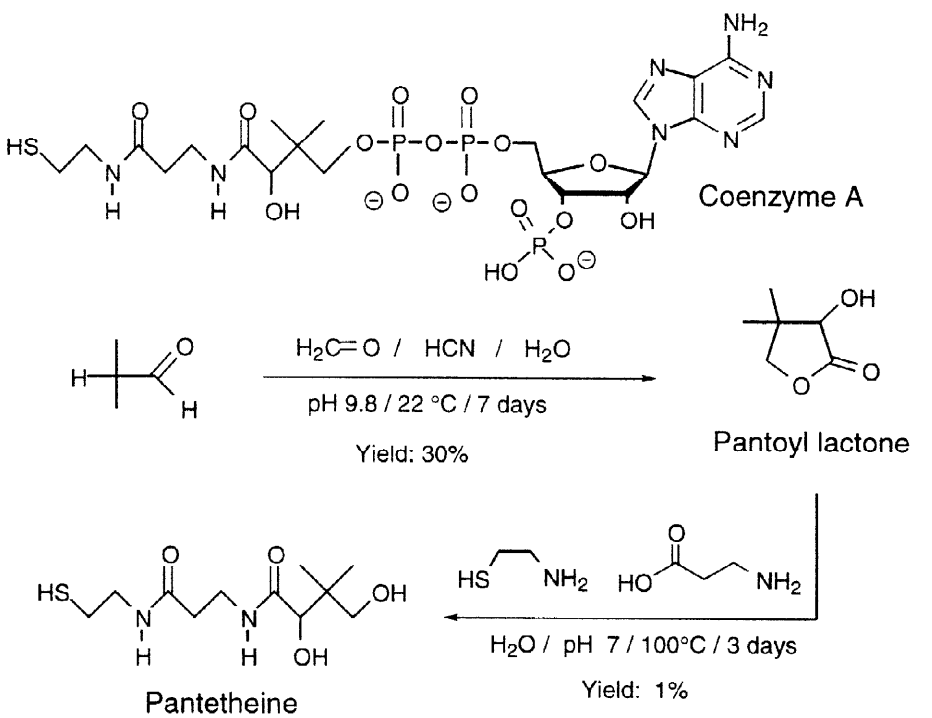



Figure 15. A possible prebiotic synthesis of pantetheine, a precursor of coenzyme A.[143,144]

### 4.4 The Case of Histidine

Histidine is one of the amino acids most often involved in the active sites of proteins. The imidazole nucleus of histidine participates in the transfer of protons required for ester hydrolysis by esterases, for example in α-chymotrypsin, for the hydrolysis of the functional amides by proteases such as trypsin and for the hydrolysis of nucleic acids by ribonucleases. It can also be a ligand of the zinc cation at the active site in carboxypeptidases, a ligand of the copper cation in haemocyanin, which transports dioxygen into certain invertebrates, and in superoxide dismutase and many other metalloproteins. The imidazole nucleus is therefore an essential element in the living world. Histidine, as opposed to the other amino acids, is very difficult to obtain *in vitro* under prebiotic conditions. Some derivatives carrying the imidazole nucleus have been formed in the polymerisation of hydrogen cyanide on the way towards adenine or guanine.

From these observations and from the complex biosynthesis of histidine ) (Figure 16), it can be considered that histidine has become involved in biological catalysis only recently and that originally its role must have been taken by another molecule (unless this biosynthesis has been preceded by an earlier and simpler one). Adenine is a good candidate for this rôle : in present day metabolism, the adenine nucleus, in the form of ATP, is one of the precursors of histidine (Figure 16). Adenine or one of its derivatives would have been able to fill some of the functions of imidazole in catalysis (see next part). The imidazole part of the purine cycle could be implicated in the transfer of protons under different cellular pH conditions from those in which imidazole functions. On the other hand, the metal cation chelating properties of adenine have been known for a long time, as opposed to the catalytic properties of its complexes which have not been studied.

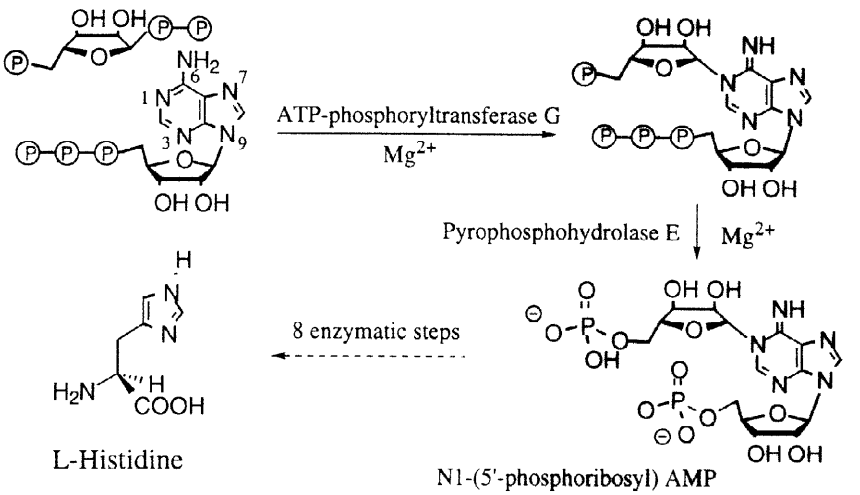



Figure 16. The first two steps of histidine biosynthesis, P = $PO_3^-$ or $HPO_4^-$.[148]

The link between adenine and imidazole is underlined by the results recently obtained on the reactivity of $NAD^+$ a cofactor also considered as fossil.[148,149] The positive charge carried by the pyridinium nucleus of $NAD^+$, makes it sensitive to hydrolysis, a reaction which involves an oxocarbenium ion intermediate (Figure 17). Heated under anhydrous conditions in the presence of sodium bromide, $NADP^+$ can be cyclised in 28% yield to give cyclic ADP-ribose (cADPR), which is involved with the metabolism of calcium ions. On the other hand cADPR can also react under abiotic conditions to give a precursor of histidine (Figures 17 and 16). This parallel between the reactivity of $NAD^+$, a cofactor considered fossil and the biosynthesis of histidine seems troubling to us. The reaction conditions used here cannot at the present time be considered as prebiotic, but the presence of a catalyst might modify these conclusions.

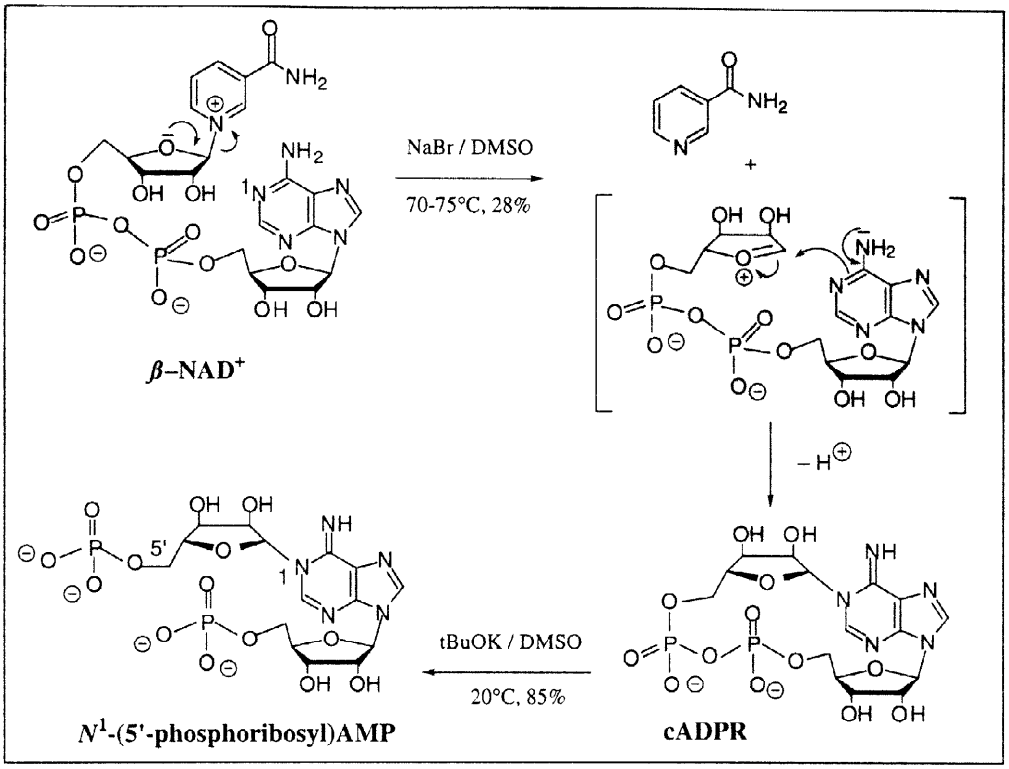



Figure 17. Decomposition of β–NAD+.[148,149]

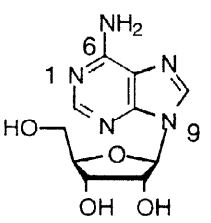
### 4.5 Adenine and its Derivatives

In natural nucleotides, the purines are linked to ribose by the $N^9$ nitrogen atom of the imidazole ring (Figure 18). RNA analogues in which the ribose is attached to another nitrogen atom of the adenine ring and thus in which the imidazole ring carries a hydrogen atom could therefore possess special properties. In this way, we have shown that a simple nucleoside, the potentially prebiotic $N^6$-ribosyl adenine (Figure 18), has a catalytic effect similar to histidine in the model hydrolysis of *para*-nitrophenylacetate.[150]

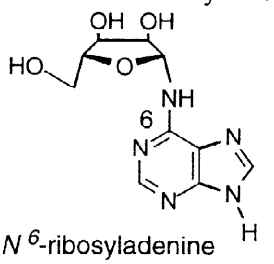
This catalytic activity is greatly increased by linking the adenine nucleus by its $N^6$-amino group to a macromolecule carrying non-protonated aliphatic amine functionality. In this favourable micro-environment, cooperativity between the amine group and the adenine nucleus is probably involved in the catalytic process.[151-153] The unusual kinetic behaviour of these macromolecules was also successfully modelled computationally.[154]

We are also studying the catalytic properties of copper(II)-adenine complexes with the aim of exploring one of the essential aspects of primitive catalysis : that of structures analogous to metalloenzymes.[94]

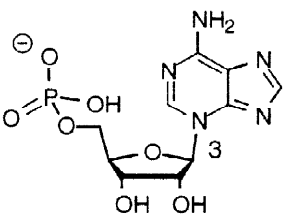
In the area of original replication, investigation of the properties of nucleotide analogues has been well developed. Orgel and coworkers showed in 1988 that 3-isoadenosine 5'-phosphate, an isomer of the normal adenosine 5'-phosphate (Figure 18), was easier to polymerise on a polyuridylic acid template than the natural nucleotide.[155] A Hoogsteen-type pairing which implicates positions 6 and 7 of the purine directs this polymerisation. The same year, Wächtershäuser proposed an original purine-purine pairing.[156] This hypothesis stems from the discovery of several cellular types of $N^3$-ribosyl xanthine (Figure 18), a purinic ribonucleoside which possesses no known function. The nucleotide is synthesised in the cell by an enzyme, a uridine-pyrophosphorylase, an enzyme specific for pyrimidines. This reaction could be the vestige of a purine-pyrimidine filiation, the purine nucleotides being the precursors. Finally, it should be noted that the mode of pairing which leaves free the imidazole ring of the purines could allow such nucleic acid analogues to exert a catalytic function.



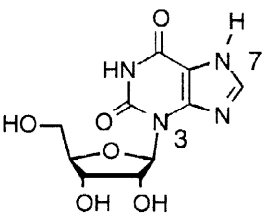
Adenosine



$N^6$-ribosyladenine



3-isoadenosine 5'-phosphate



$N^3$-ribosylxanthine

Figure 18. Structures of ribosyl-adenine[77,78,155] and ribosyl-xanthine derivatives.[156]

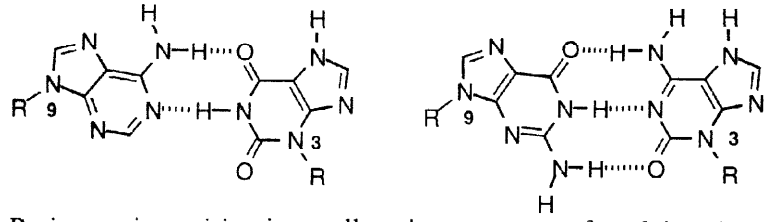


Figure 19. Purine-purine pairing in an all-purine precursor of nucleic acids proposed in 1988 by Wächtershäuser. R represents a backbone moiety derived from ribose, deoxyribose or a ribose precursor.[156]

The study of the properties of $N^1$, $N^3$ and $N^6$-(and potentially $N^7$)-substituted derivatives of purines therefore appears a wide and promising field of investigation. These derivatives may have played an important role in the molecular development of life.

## 5. Conclusion

The "RNA World" scenario of the origin of life resulted from the careful examination of the present metabolism.[53,54] In this "world", an ancestor of RNA, a common precursor to all forms of life, catalysed the necessary reactions to assemble and replicate itself and for life. The discovery of ribozymes showed that present RNAs possess catalytic properties. Recently, methods have been developed for generating and screening large libraries of nucleic acid molecules differing in their sequences and their folding properties.[55-60] Ribozymes capable of assembling short oligonucleotides or performing the same peptidyl transferase reaction as the ribosome for linking amino acids during protein synthesis were selected. The success of these methods in the selection of RNAs possessing new catalytic properties and RNAs that bind strongly bioorganic molecules such as amino acids, ATP or enzymatic cofactors lend experimental support to the "RNA World" hypothesis .

The range of activities of RNAs, already quite wide, must therefore be extended to the catalysis of new reactions. The primitive nucleotides could have been different from the nucleotides found today. It is therefore important to study all the properties of nucleic acid analogues in which not only is the ribose-phosphate backbone modified but also the bases and in particular the purines susceptible after modification to display catalytic activities.

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## Figures

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## Biographical sketch

Marie-Christine Maurel

Jean-Luc Décout

Guy Ourisson

Yoichi Nakatani

Marie-Christine Maurel was born in 1948 in Nice (France). Since receiving her Thesis from Paris 6 University in 1985, developping a method for detecting tertiary structure in RNA, she has been performing post-doctoral research about Origins of Life in Dr. Jacques Ninio's laboratory, specially studying nucleic acids-like structures and activities. Now as Professor at Paris 6 University (Pierre and Marie Curie), she is involved in testing RNA world hypothesis. She also develops research on peptide synthesis after a recent six months stay in Dr. Leslie Orgel's laboratory at the Salk Institute.

Jean-Luc Décout graduated from the University of Lille in polymer photochemistry and in bioorganic chemistry. After one year as a postdoctoral fellow with Professor K. K. Ogilvie at McGill University in Montreal, he joined the University of Grenoble in 1987. He is now Chargé de Recherches in the CNRS, in the Department of Molecular and Cellular Biology (DBMS) at the Centre d'Etudes Nucléaires in Grenoble. His research interests include prebiotic chemistry and the design and the synthesis of biologically relevant molecules, especially modified nucleosides, nucleotides and oligonucleotides.

Guy Ourisson, born in 1926, has been on the staff of the University of Strasbourg since 1955. He was the founding President of the Louis Pasteur University in the early 70's, and in the mid-80's the successor of Sir Derek Barton as the Director of Institut de Chimie des Substances Naturelles in Gif-sur-Yvette. He is presently the Vice-President of the Académie des Sciences.

With his associates from more than 30 different countries, he has established the structures of many natural products (longifolene, the gurjunenes, trachylobanic acid, shionone, frullanolide, etc.), initiated the use of plant tissue cultures for biosynthetic studies, launched the large scale study of biomarkers from sediments which led to the discovery of the important class of geohopanoids and of their bacterial precursors the biohopanoids, and is now engaged in the study of the evloution of membranes and of the emrgence of cellular life.

He has been an Editor of Tetrahedron Letters since 1967.

Yoichi Nakatani, born in 1937, got his Doctorate in 1960 from the University of Tokyo, under the leadership of Professor Matsui. He remained there as an Assistant, until he joined Professor Ourisson's group in 1967 - 1968. He returned to Tokyo where he got an Assistant-Professorship at the Ochanomizu University from 1968 to 1978, and studied extensively the structures of some characteristic aromas such as green and black tea, sesame oil, citrus peel oil, etc.. In 1978, he returned definitively to Strasbourg, and has since collaborated with Prof. Ourisson as a CNRS senior staff member and a guest Professor at Université Louis Pasteur. In recent years, he has concentrated fully on the study of primitive membranes.