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Chapter 6
Scenario of the Primary Pump: Emergence and Operation of an Automatic Engine to Generate Primordial Peptides and Beyond Nucleic Acids

Auguste Commeyras

Abstract The emergence of peptides and nucleic acids is a major concern in prebiotic chemistry.

Using water as solvent, the most practical way to form peptides and nucleic acids by dehydration reactions is to use amino acids and nucleotides in their activated forms. One is then faced with activated compounds considered so sensitive to hydrolysis that their prebiotic relevance is frequently questioned. Thus, the prebiotic synthesis of such macromolecules remained a wide open problem.

By analyzing how living organisms work, one finds that the peptides and nucleic acids are, in fact, synthesized in aqueous medium from activated amino acids and nucleotides. Here, it is crucial that the living world is constantly kept out of equilibrium. We then asked, according to the "principle of evolutionary continuity," whether the environment of early Earth could have favored the spontaneous emergence of a particular chemical non-equilibrium system, which could have been the source of macro-chemical evolution. We have identified such a system, together with operating conditions necessary to keep it out of equilibrium. We have called this energizing process the "primary pump."

We detail its operating mechanism and evaluate its credibility. We show how this primitive pump was running continuously for a long geological period in which it could maintain disequilibrium in a particular chemical system of complex interactions. We show how the amino acids could be concentrated, how they were condensed in a dehydration reaction, how the primary pump has been able to select certain monomers during the condensation steps, and how the primary pump may

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have caused the evolution of the peptides formed. We point out how the primary pump could have transferred its energy to other reactions, such as taking part in the appearance and evolution of nucleic acids. The "scenario of the primary pump" is a dynamic process through which peptides could have emerged and, perhaps, co-evolved with nucleic acids – two interactive macromolecules fundamental to life.

6.1 Why a Primary Pump?

6.1.1 The Principle of Continuity Requires a "Primary Pump"

Life is a chemical system out of equilibrium, a dynamic system that continuously receives energy. This energy is the driving force of evolution. On the primitive Earth, the elementary molecules (CO_2, H_2O, N_2, . . .) have been gradually transformed into essential-for-life molecules (amino acids, nucleotides, . . .) through energy provided by sunlight, lightning (in thunder storms and volcanic plumes),[1] and entry of meteorites into the atmosphere (Commeyras et al. 2005a, b).

In the primary pump scenario, we postulate that essential molecules could have been assembled, organized, and maintained out of equilibrium by a constant supply of energy.

We believe that such a dependable power pack had spontaneously emerged on the "primitive Earth." We have called this permanent provider of energy the "Primary pump," in reference to its primordial origin and function. The purpose of this chapter is to seek a process, compatible with the environment of the early Earth, which could gather all the requirements desired.

6.1.2 The Primary Pump

A pump is a machine that transports material uphill against an energy difference. It is characterized by: Energy consumption, a movement (rotation, flap), and a non-return valve. Life is associated with many pumps. A beating heart is life (hearts have non-return valves). If the heart stops beating, it means death. In a pump, energy is provided by oil, by electricity, by food, and, ultimately, by ATP in

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[1]The energy of sunlight is unable to dissociate, N_2 into radicals N. This energy is only found in lightning (in thunder storms and volcanic plumes) or entry of meteorites. The radicals N then give nitric oxide (NO) in the excited state (singlet). The transition, singlet state to normal state, emits the light of lightning and meteorite trails. In volcanic plumes, NO production is very high due to direct reaction and almost continuous volcanic lightning (Navarro-Gonzalez et al. 1998; Mather et al. 2004). On the primitive earth (with a small amount of O_2), the nitrosating agents, once formed, were stable. Today, with a large amount of oxygen in the atmosphere, NO is quickly transformed into NO_3H.

biological pumps. Obviously a primary pump has been very simple, but what could have been its source of power, its principle of operation?

6.1.3 Darwin and Evolution

Life has a history. Today, the Earth numbers between 5 and 100 million different organisms. One billion years ago, life was certainly very primitive and deregulated, and essentially all the organisms should have been "interbreeding."

Scientists believe that 2.5 or 3.5 billion years ago, there should have lived a peculiar species, the "Last Universal Common Ancestor" (LUCA), from which all modern organisms have descended. It is well before LUCA that we must ask the question of the origin of life. With the appearance of the stage of LUCA, Darwinian evolution[2] was under way. Long before LUCA, the inert material was progressively becoming more and more complex until reaching the LUCA stage. When inert matter had been able to reproduce, it jumped into a new world – unknown and extraordinary.

What is the origin of this common ancestor? We believe that LUCA, like modern organisms, necessarily depended on a chemical energy pool that allowed it to survive and evolve. Whether the heart of this chemical energy currency was ATP is still an open question (Gargaud et al. 2009). If the pumps for LUCA were similar to those that enable the life of bacteria, they should have been very effective. Indeed, to stay alive, bacteria concentrate millions or even billions of times the nutrients of their environment.

Long before LUCA, the concentration of reactants in the primitive ocean was extremely low (Sect. 6.2.1). Accordingly, for the primary pump to be effective (taken as a machinery to complexify the inert prebiotic matter), it had to implement very efficient processes to concentrate its reagents. Searching for an appropriate concentration process was one of our concerns throughout this work.

6.1.4 What is the Environment that Led to the Birth of LUCA?

Very briefly, the environment of the pre-LUCA era can be summarized as follows. The Earth was born 4.56 billion years ago. The Earth's crust (floating on the upper mantle) was formed very early (between 4.55 and 4.3 billion years ago). The rocks of the crust were mainly basaltic and very hot (Tessalina et al. 2010). The water (remaining from planetesimals and brought in by comets) was vaporized initially (clouds). The atmosphere was reductive and rich in CO_2 (~10 bars). The water in the clouds was acid (carbonic acid H_2CO_3 pH 3.5). During the cooling phase, oceans

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[2]There is no reason to believe that LUCA as such was the start of Darwinian evolution. It just happens to be the stage from which more than a single line of descendents have survived to modern times (Forterre 2005). We simply have no way of knowing how many other lineages from pre-LUCA stages have all gone extinct.

were formed. Alkali metals (e.g., sodium) were easily extracted from basaltic rocks. The pH was then controlled by the buffer carbonic acid/sodium bicarbonate. When subsequently calcium was extracted, limestone precipitated and concentration of CO_2 decreased in the atmosphere, yet stabilized at intermediate levels.[3] Just like today, the oceans did not cover young continents completely (Gargaud et al. 2009), although the total mass of continental crust was much smaller early on.

Eighty million years after its formation (that is to say, very early), Protoearth collided with the protoplanet Theia, a Mars sized protoplanet in the same orbit. This was a kind of cosmic reset. The Moon was created by this collision. The Moon was originally closer to the Earth of about 70,000 km (today it moves away 1 cm/year). It was revolving around the Earth, not in 28 days as present, but in about 17 days. With the Moon nearer, Earth's tides were greater than today. With a rotation speed faster (day length was ~50–75% its present value), the frequency of the tides was higher than today. The early oceans, probably washed with high efficiency, young continents, pulling their soluble portion.The early Earth resembled a gigantic washing machine (Lathe 2004; Varga et al. 2006). The high impact rate of cosmic bolides after the Moon-forming event subsided rather slowly, and even reached peak values once again during the so-called late heavy bombardment phase (Gomes et al. 2005). Life has emerged in such an energized environment.

6.2   Primary Pump: The Challenges

6.2.1   Peptide Synthesis

Although knowledge in this area is not definitive, it is considered that the primitive earth might have carried about hundred racemic amino acids. Their concentration did not exceed a few mg/l[4] in an ocean with a pH between 5 and 6 (Commeyras

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[3]Only after Life and oxygenic photosynthesis had emerged, could CO_2 concentration drop to modern levels, as driven by both organic carbon sequestration and biogenic acceleration of limestone precipitation, mainly due to pH increase in the oceans.

[4]An exhaustive inventory of the origins of all the organic molecules occurring on the primitive Earth 4 Giga years ago was carried out by Chyba and Sagan (1992). Three probable sources were distinguished: exogenous contribution, endogenous synthesis associated with impact of meteorites, endogenous production associated with other available energy sources (solar UV, lightning).The total contribution of these three sources would have been heavily dependent on the composition of the primitive atmosphere. The primitive oceans could have contained (at a steady state) between ca. $0.4\ 10^{-3}$ g/l of total organic matter in the case of a neutral atmosphere and 0.4 g/l in the case of a reductive atmosphere. The organic matter carried out by carbonaceous chondrite was mainly (>60%) macromolecular, practically insoluble, and characterized as PAHs with few or no impact on the origin of life, but who knows? (Deamer, 1992). The soluble part (<40%) is a complex mixture of molecular organic compounds in which the following have been characterized: acids (carboxylic, dicarboxylic, hydroxyl, sulphonic, phosphonic); amines; amides; nitrogenous heterocycles including purines and pyrimidines; alcohols; sugars; carbonyl
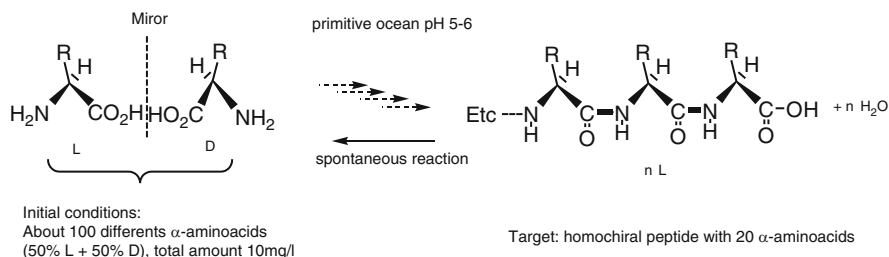
Fig. 6.1 Homochiral peptide from initial conditions

et al. 2005a, b). Taking into account all of these initial conditions, no previous work on the emergence of prebiotic peptides (Fig. 6.1) has been undertaken, certainly because these conditions have been a priori considered to be very unfavorable to such syntheses.

If we venture in this direction, the first classic reflex is to decompose the problem into a series of basic questions such as:

1. Is it necessary to concentrate the amino acids in order to condense them?
2. Is it necessary to activate the amino acids, and if so how and where to be able to condense them?
3. If peptides are formed, could they evolve via a reaction loop: hydrolysis-condensation?
4. Can we imagine a prebiotic process to repeat such a cycle?
5. Can we consider that the above cycle could be responsible for chemo- and enantio-selection?
6. What process can we imagine to transform a racemic mixture of α-amino acids into a homochiral peptide?
7. How can the emergence of catalytic properties of peptides be imagined?

Thus, we are faced with a series of questions, which are difficult to consider independently. It is indeed implausible to imagine that processes as diverse as those involved in the questions above, have worked separately. Had that been the case, the emergence of homochiral peptides would have been solved by chance.

Our group did not want to follow this reductionist approach. We have instead preferred to be interested in a holistic approach, focusing on the scenario, rather than the actors. Like the chemical energy that keeps the cells alive and out of equilibrium with a capability of evolution, we devised a primitive machinery that could have provided an answer to all of the issues raised by the emergence of peptides.

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compounds; aliphatic and aromatic hydrocarbons; amino acids; and even hydantoins. The average mass of extracted amino acids was only 0.6 μg per gram of carbonaceous meteorite. Only the amino acids are taken into account in the first part of this document.

It is this machinery that we have called "primary pump" (Commeyras et al. 2002). We designed it as a reaction cycle, repeated continuously. The primary pump has two different aspects: chemical reactions and mechanical/material transport.

6.2.2   The Primary Pump: Chemical Part

To see the primary pump in its environment go to Sect.. 6.3.

The following four reactions are the chemical components of the primary pump.

Step 1: Activation of amino acids,

Step 2: Polymerization of activated amino acids to create peptides,

Step 3: Epimerization of the last amino acid incorporated into the peptide chain to give the configuration of the preceding amino acid,

Step 4: Depolymerization of the peptide chain formed to split it into shorter peptides.

The potentially prebiotic qualifications of these reactions are discussed below.

Step 1: Activation of α-amino acids

The reaction of a gaseous mixture NO/O_2[5] on anhydrous N-carbamoyl-amino acid gives equimolar amounts of N-carboxyanhydride of α-amino acids (NCAs), water, and nitrous acid (Collet et al. 1996). The NCAs are activated α-amino acids. They are potentially prebiotic (Pascal et al. 2005). The gas mixture NO/O_2, as generated by energetic processes in the atmosphere, is also potentially prebiotic (Commeyras et al. 2002). The N-carbamoyl-amino acids can prebiotically be obtained in two ways (Fig. 6.2), by hydration of hydantoins or carbamoylation of α-amino acids (Commeyras et al. 2005a, b).

Step 2: Polymerization of NCAs

The polymerization reaction of NCAs to provide peptides is known to work. We have shown that this reaction may be performed in water as solvent, provided that the pH is above 4, which could have been the case of the primitive ocean (Commeyras et al. 2005a, b; Pascal et al. 2005; Collet et al. 2010).

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[5]In prebiotic conditions, the abiotic oxygen, at very low partial pressure, was essentially formed by photo dissociation of CO_2 in upper atmosphere. Nitric oxide (NO), at much higher partial pressure than O_2, was formed by lightning and meteoritic impact in lower atmosphere. The meeting of NO and O_2 gives (N_2O_3, NO_2, N_2O_4) in equilibrium. The O_2 concentration is low in the mix NO/O_2 (at all likelihood that was the case in prebiotic conditions) while the concentration in N_2O_3 is strong. And precisely, N_2O_3 is the powerful nitrosating agent. It reacts with N-carbamoyl amino acids in dry phase with a very high kinetic constant ($k_{N2O3} = 1.0 \times 10^8 s^{-1}$) (Lagrille et al. 2007), to give NCA, water, and HNO_2 in equivalent amounts. Under the same conditions, N_2O_3 causes decarbamoylation of peptides (see animations 1 and 2).
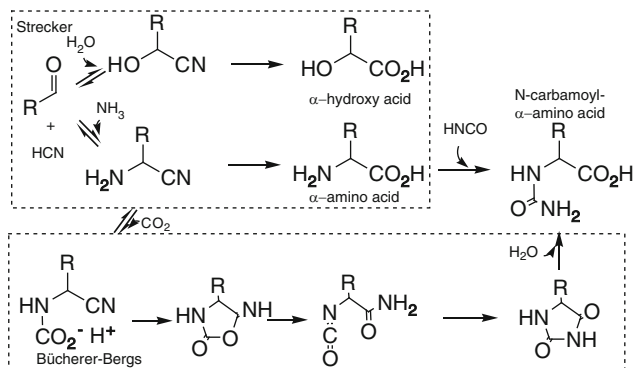
Fig. 6.2 N-carbamoyl-amino acids from two different pathways, Strecker in extraterrestrial conditions, Bücherer-Bergs on early Earth

Step 3: Epimerization[6]

Epimerization of the last amino acid incorporated into a peptide chain to give the configuration of the previous amino acid is less documented than the steps 1 and 2. It is a catalytic reaction (Honnoraty et al. 1995, Plasson et al. 2004). It could be effective when the peptide chain adopts the helical conformation, that is to say beyond a length of about 10 mer. Further work must be done to develop knowledge in this field.

Step 4: Depolymerization

The depolymerization reaction of a peptide chain is slow (Radzicka and Wolfenden 1996, Smith and Hansen 1998). In water at pH between 4 and 7, the half-life time of this reaction is 400 years, compatible with prebiotic conditions and the primary pump scenario. If the hydrolysis rate is faster than the rate of formation of peptides, the process to be considered is counterproductive. If instead, the rate of hydrolysis is slower than the rate of elongation and preferentially occurs in unfolded regions, the process leads to the accumulation of foldable peptides in the reaction medium. In consequence of partial hydrolysis and a lengthening of fragments, the peptides formed were forced to evolve (Commeyras et al. 2005a, b). In particular, if the rate of chain elongation is slightly autocatalytic, requiring a certain length to be most effective, partial hydrolysis of the longest peptides could increase the number of growing chains substantially.

During these cycles, while some amino acids are incorporated more rapidly than others, chemo-selection results in the peptide chain (Commeyras et al. 2002, 2004a,b). Similarly, if one of the two D- or L-enantiomers is more rapidly incorporated than the other in the peptide chain, this is resulting in enantio-selection (Commeyras et al. 2004a, b). The enantiomer not incorporated in the

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[6]For the definition of this term, see wikipedia.

chain racemizes slowly in the primitive ocean (Csapo et al. 2004) and the process continues. The result is a total transformation of the initial stock of racemic amino acids into a homochiral peptide. To understand these phenomena of chemo-selection, enantio-selection, and deracemization, we refer the reader to the work of Raphael Plasson who has developed experimental and kinetic models which describe the dynamic operation of the primary pump (Plasson 2008; Plasson and Bersini 2009; Plasson and Brandenburg 2010; Plasson et al. 2004, 2007, 2010).

6.2.3 The Primary Pump: Mechanical Part

As mentioned above, the concentration of α-amino acids in the early oceans was very low, certainly lower than 10 mg/l (Commeyras et al. 2005a). In water and at such low concentrations, the dehydration reactions are impossible.

For the primary pump to operate, the following conditions must be met:

- The N-carbamoyl-amino acids must be synthesized preferentially (Taillades et al. 1998, Commeyras et al. 2005a,b), somewhere in a patchy environment.
- The N-carbamoyl-amino acids must be concentrated to become anhydrous.
- The anhydrous N-carbamoyl-amino acids must be subjected to the action of a gaseous mixture NO/O_2 (N_2O_3) to form N-carboxyanhydrides (NCAs), water, and nitrous acid whose pH ~ 1.5.
- The pH of the environment of NCAs must be suddenly changed to become less acidic (pH > 4).
- All reactions of this cycle must occur under natural conditions.
- All reactions of this cycle must be repeated continuously.

For such a logical sequence to operate effectively, the primary pump must have a mechanical component. The mechanical means of interest must be compatible with the natural environment of the primitive Earth. Indeed, all these conditions could have been observed at surfaces close to the triple junction of ocean, continental crust, and atmosphere. That is to say on the "beaches" of a primitive continent, and nowhere else.

Figure 6.3 summarizes these conditions.

The dotted line represents the boundary between the ocean and continents.

- In the ocean at pH 5, N-carbamoyl-amino acids were formed in two different ways as we have seen previously.
- At high tide, the water inundates the land surface with a batch of diluted material. Upon withdrawal, evaporation concentrates the N-carbamoyl-amino acids to the crystallization point. Thus, evaporation could have been the first natural technique to concentrate the starting material of the primary pump.
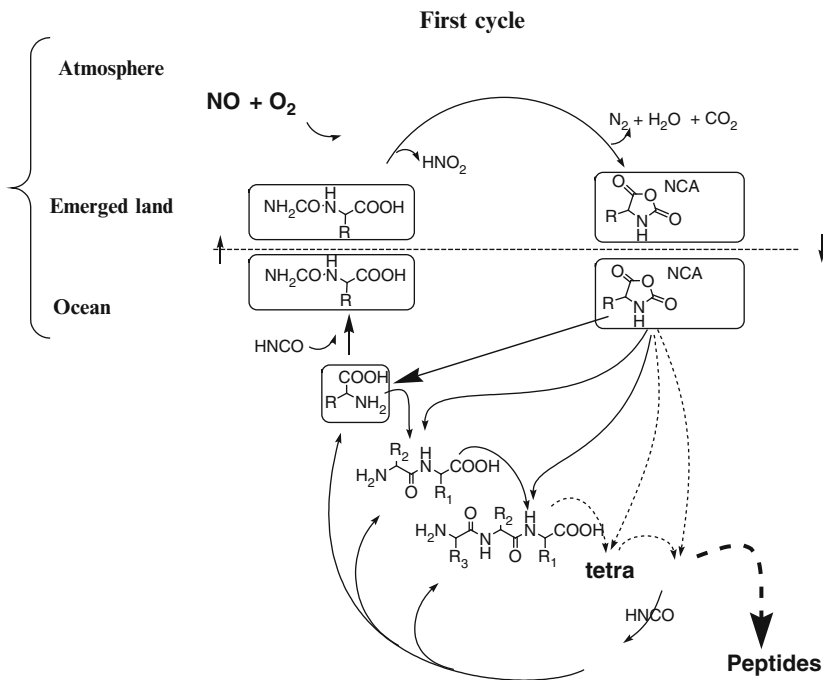
Fig. 6.3 Diagram of the primary pump – first cycle, idealized

- The atmospheric gas mixture (NO-O_2) reacts on the anhydrous N-carbamoyl-amino acids, and transforms them into NCAs (Commeyras et al. 2004a). The acidic environment due to nitrous acid (pH ~ 1.5) stabilizes the NCAs.
- At the next high tide, the NCAs are suddenly covered by the ocean at pH > 4. They polymerize to give peptides.
- All reactions of this cycle are natural.
- This cycle is repeated continuously with the frequency of the tides.

6.2.4 Details Giving Credit to the Primary Pump

Isocyanic acid (HNCO) can react on the emerging peptides to give N-carbamoyl-peptides (H_2NCO-Peptide). In this way, the peptides are protected. With such protection, peptides cannot be elongated and the primary pump loses its prebiotic credibility. For this not to occur, the protection must be removed.

In considering this question, we showed experimentally that in the presence of the gas mixture NO/O_2 the protection is immediately removed. This reaction produces nitrogen and carbon dioxide (Collet et al. 1999). We noted that the conditions to remove this protection are strictly identical to those that allow
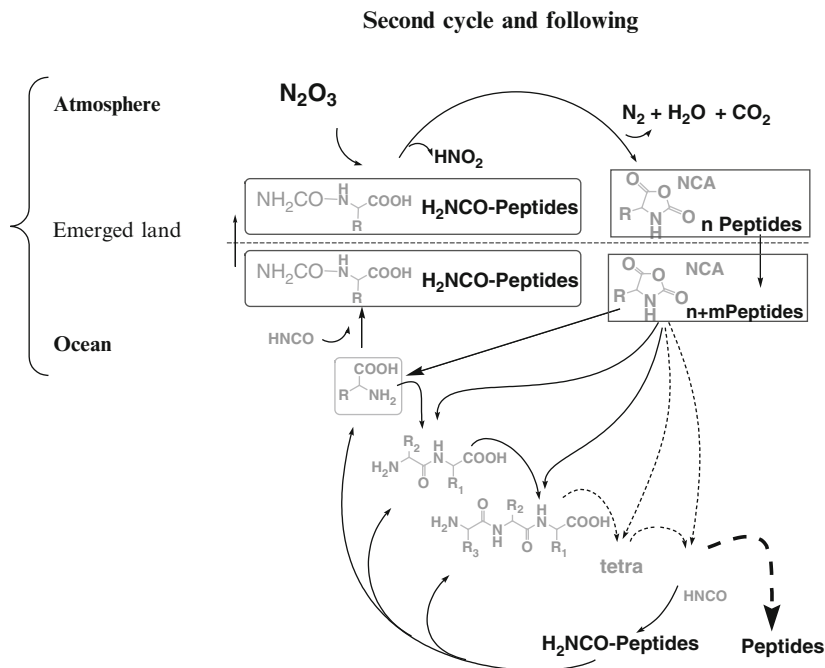
Second cycle and following

Fig. 6.4 Diagram of the primary pump – recurrent cycling

N-carbamoyl-amino acids to give the NCAs, so the free peptide obtained must be elongated during the same cycle, and the primary pump can continue to run (Fig. 6.4). The conditions for this deprotection, and the total recycling of matter, appeared to enhance the credibility of this scenario.

6.2.5 The Primary Pump: Myth or Reality?

The reaction cycle of the primary pump can only turn in one direction. (The main steps are indeed irreversible). This reaction cycle draws on a stock of racemic α-amino acids and (ideally) assembles them into a stock of homochiral peptides. With a high speed (Two cycle per ancestral day), homochiral peptides accumulate in the environment. The energetic pressure of the primary pump maintains the "homochiral peptides" out of equilibrium (Plasson and Bersini 2009).

For the stock of homochiral peptides to return to equilibrium, the only possibility would be to stop rotation of the earth, thus eliminating the mechanical component of the primary pump. The new equilibrium, after several hundred years (Csapo et al. 2004), would be characterized only by the presence of racemic amino acid and no

peptides. Obviously, such a scenario is impossible. This could mean that, after having started, the evolutionary process had been irreversible.

In summary, the primary pump appears to be a scientifically acceptable tool to study how inert matter could have been complexified, even as some gray areas will remain to be clarified.

The operation of the "primary pump" is visualized by the following cartoon.

Animated scheme. Primary pump in motion {link: Animation 1}

6.2.6 How Many Peptides Possible?

The number of peptides that the primary pump can assemble is given by the relation $2 * Y^X$, where 2 are the D- and L-enantiomers of α-amino acids, Y is the number of available amino acids, and X is the number of monomer in the peptides. Initially, with about one hundred α-amino acids and two enantiomers the number of peptides that the primary pump could make was huge. After chemo- and enantio-selection, this number was lower ($1 * 20^X$) but nevertheless significant. Insofar as the reaction cycle (pump) has played a role in the history of the Earth, it took a creative power, and selection, unparalleled in the world of chemistry. Modern life has retained a number of peptides much lower than theoretical possibilities. Nothing is known of the selection processes that have been implemented.

6.2.7 The Primary Pump Yesterday and Today

The primitive Earth atmosphere was reducing (Gargaud et al. 2009). Traces of oxygen were formed in the upper atmosphere (Commeyras et al. 2002). The activation of the lower atmosphere, by photoactivation, lightning, volcanic plumes, and meteor entry, produced nitric oxide (NO), which together with traces of oxygen gave nitrosating agents such as N_2O_3 (Lagrille et al. 2007). These nitrosating agents, with isocyanic acid, were the source of chemical energy for the primary pump. To this chemical energy was added the mechanical energy of ocean tides (cosmic energy).

Today, the Earth's atmosphere contains 21% oxygen. This oxygen instantly transforms nitric oxide into nitric acid. The nitrosating activity is then immediately lost. The primary pump cannot work anymore. We must therefore consider that the primary pump ceased working with the gradual arrival of the oxygen in the atmosphere, accordingly with the emergence of life (the "takeover") and biogenic photosynthesis. Life has created the poison (O_2) which, in turn, could make it disappear. Fortunately, evolution was saved by allowing living cells to adapt, so as

to operate at relatively high proportions of oxygen. Darwin's principle (i.e., natural selection) was, of course, already present from the start.

6.3   The Primary Pump in its Environment

6.3.1   The Primary Pump has not Remained Isolated in its Environment

The experimental information presented below shows how the primary pump has shared its energy with its environment. In summary of these experimental data, Fig. 6.5 locates the primary pump at the heart of an idealized device that can describe how the original organic material could have continued to evolve. We have represented the Sun, the Earth with its atmosphere, and the Moon. The atmospheric layer of about 10 km thickness played a crucial role in promoting the emergence of life.
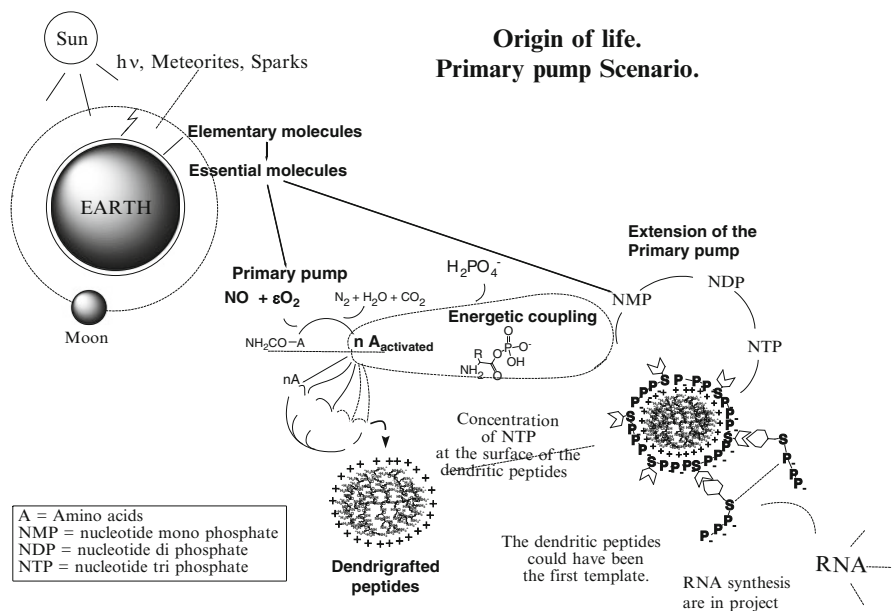
Fig. 6.5 Overall presentation of the primary pump scenario: Activation of the primitive atmosphere gives activated molecules. These activated molecules give the molecules essential to life. The primary pump, continuous turn's amino acids into amino acids activated (NCAs). Parts of NCAs give dendritic peptides. Another part reacts with phosphoric acid and then with nucleosides to form the NTPs. The NTPs concentrate and stabilize with the dendritic peptides. Beyond that, the first oligonucleotide may have formed

6.3.2   From Elementary Components to Essential Bio-Molecules

The primitive atmosphere (N_2, CO_2, CH_4, H_2O, rare gases), excited by solar radiation, lightning, volcanic plumes, and meteor entry, has produced energy-rich molecules HNCO, HCN, NO, O_2, HCC-CN H_2N-CN, RCHO, $HOCH_2CHO$, $HOCH_2CHOHCHO$ . . . (Gargaud et al. 2009). These activated precursors have led to essential bio-molecules, such as amino acids or nucleotides (Commeyras et al. 2005a, b; Powner et al. 2009).

6.3.3   α-Amino Acids

Seventy-four different α-amino acids have been identified in the Murchison meteorite (Cronin and Pizzarello 1983, 1997); they were formed via the Strecker reaction (Commeyras et al. 2005a, b), so it is reasonable to think that some amino acids on the primitive Earth were of exogenous origin; but we believe that a much higher proportion of α-amino acids was probably formed on the primitive Earth itself, through a Bücherer-Bergs reaction (Taillades et al. 1998, Commeyras et al. 2005a, b).

The difference between the two processes comes from the presence of high proportions of CO_2 in the primitive atmosphere.

Strecker reaction:

RCHO + HCN + NH_3[7] → α-amino acids.

Bücherer-Bergs reaction:

RCHO + HCN + $CO_3(NH_4)_2$ → Hydantoin → N-carbamoyl amino acid → NCA → peptides.
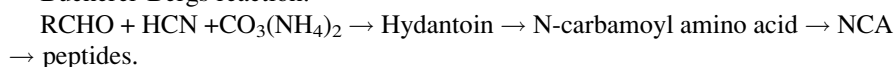
These two mechanisms have a common part, and then they diverge (Fig. 6.2).

We represent below, in motion, the production of peptides from activated molecules.

Animated scheme.   Peptide synthesis from activated molecules {link: Animation 2}

Through the Bücherer-Bergs reaction, the first stable products formed are the hydantoins. The hydration of hydantoins gives N-carbamoyl-amino acids. Then the reaction of the gas mixture NO/O_2 on N-carbamoyl-amino acids gives the NCAs. In water at pH > 4, NCAs lead to peptides. During the formation of peptides, CO_2 is regenerated and returns to the atmosphere.

This animation shows the existence of a "short link" between the primitive atmosphere and peptides. In a supportive environment, a primitive atmosphere leads to peptides with minimal steps. All responses are geologically rapid (some days), and these reactions are repeated every day. The synthesis of peptides on the primitive Earth might therefore have been very fast.

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[7]We must note that ammonia (NH_3) was probably formed in the oceans by NO reduction by Fe^{2+} (Summers and Chang 1993).

6.3.4   Structure of the First Peptides, and Their Potential Role in Evolution

The first emerging peptides from polymerization of NCAs cannot have been encoded for particular sequences, since the genetic code had not yet emerged. The likely presence of polyamine or thio-amino acids in the reaction medium was probably used as cross-linking agents and led to the formation of dendrigraft amino acid condensates.[8] Such globular structures have polycationic surfaces. Depending on their hydrophilic/hydrophobic balance, they remained soluble in water up to very large molecular weight (~10^6 Da) (Collet et al. 2010). Some of them have necessarily been adsorbed to the polyanionic surface of rocks and finer grains of sediment. These dendrigraft peptides, soluble or adsorbed, could have played the role of micro-reactors in chemical evolution. We will discuss this aspect below.

6.4   Emergence of Oligonucleotides

6.4.1   Prebiotic Synthesis of Nucleotides

Similar to peptide formation, the synthesis of nucleic acids from nucleotides is a dehydration reaction (Fig. 6.6). This applies to modern metabolism and prebiotic considerations alike.

John Sutherland and colleagues have recently shown that it is possible to synthesize pyrimidine ribonucleotides from activated elementary molecules (Fig. 6.7) (Powner et al. 2009).
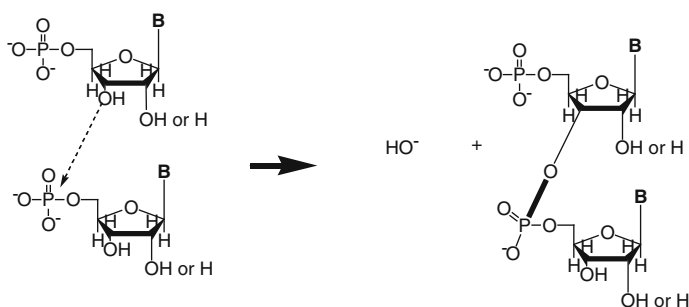

Fig. 6.6 Formation of sugar phosphate bonds, formally by a dehydration reaction

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[8]Dendrigraft polymers are a class of highly branched macromolecules belonging to the dendritic polymer family. Multiple branching levels characterize the architecture of these molecules, in analogy to dendrimers and hyperbranched polymers (Teertstra and Gauthier 2004).
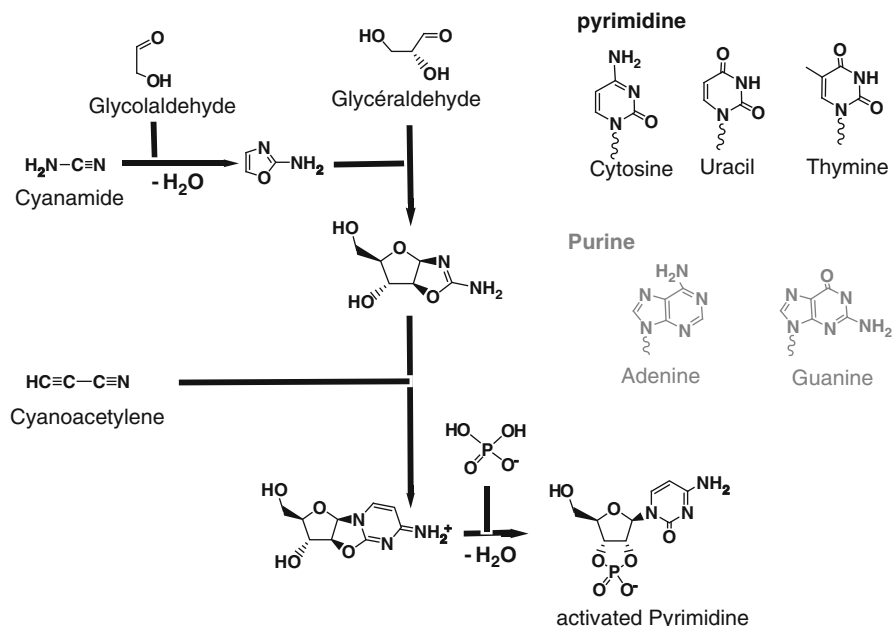
Fig. 6.7 Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions

Extrapolating from these results, we here assume that a group of ribonucleotides could have been formed on the primitive Earth. We will not discuss the order of appearance of RNA or DNA, but only how nucleic acids could emerge. The authors involved in this research (Fernando and von Kiedrowski 2007) concluded that at least three conditions are necessary to form these macromolecules:

1. The nucleotides must be activated.
2. The activated nucleotides must be concentrated.
3. The activated and concentrated nucleotides must be positioned on templates, to promote their dehydration.

We will only take an interest in finding potentially "prebiotic" solutions to these three requirements.

6.4.2 Activation of Nucleotides

6.4.2.1 What Activation Mode for Nucleotides?

The activation via the triphosphate form (NTP) is used by living systems to produce nucleic acids. These syntheses are enzymatically catalyzed. Under abiotic conditions, attempts to oligomerize the Adenosine TriPhosphate (ATP) failed, the rate of hydrolysis of ATP being faster than its condensation. Using imidazole as

activating agent and at concentrations at least equal to 0.015 M, oligonucleotides up to 40 monomer units were obtained in the presence of montmorillonite (Prabahar et al. 1994). The prebiotic relevance of such a mode of activation of ATP has been seriously questioned. (Shapiro 2006).

Following the primary pump scenario, we have revisited this issue. Two sets of accepted data jumped to our mind:

1. ATP is universally used by the living world.
2. The living world uses a variety of metabolic pathways to produce ATP.

These data may give rise two different interpretations:

1. Perhaps, triphosphates were the first form of activation of nucleotides, and later during evolution, production modes of triphosphates have diversified (i.e., lost and gained independently).
2. Alternatively, primitive nucleotides have not been activated as triphosphates in the beginning, but in a different way which remains to be discovered. Subsequent evolution has repeatedly discovered (exapted[9]) triphosphates as a means of activating nucleotides; this is why the modes of triphosphate production are different from one species to another.

What looks illogical in the second interpretation is to imagine that evolution has universally imposed this mode of activation with a retroactive effect. We therefore considered the first interpretation as potentially more reasonable and scrutinized the scenario of the primary pump for potential impact on additional reactions:

1. A primitive process to form nucleoside triphosphate (NTP)
2. A primitive process to concentrate NTP
3. A primitive process to condense NTP, giving the first strands of RNA and (or) DNA

6.4.2.2   The Primary Pump and the Synthesis of NTPs

As we have seen before, peptides are formed when the NCAs produced in dry phase are poured into water at pH > 4. If phosphoric acid is present, at pH between 4 and 8, amino acyl phosphates are obtained as is shown in Fig. 6.8 (Biron and Pascal 2004). The amino acyl phosphates obtained in 15 min hydrolyse completely in 1.5 h. This hydrolysis leads back to amino acids and phosphoric acid.

However, if alcohols or acids are present (in the same range of pH 4–8), these compounds are phosphorylated by aminoacylphosphate (Fig. 6.9). For example, methanol gives methyl phosphate. Nucleoside Mono Phosphate (NMP) gives Nucleoside Di-Phosphate (NDP). Nucleoside Di-Phosphate (NDP) gives Nucleoside Tri-Phosphate. The ATP formed has been characterized by photon emission in

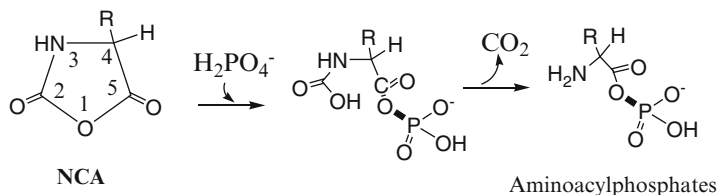---

[9]Exaptation: see wikipedia.

Fig 6.8 Amino acid N-carboxyanhydrides as phosphate-activating agents in aqueous solution
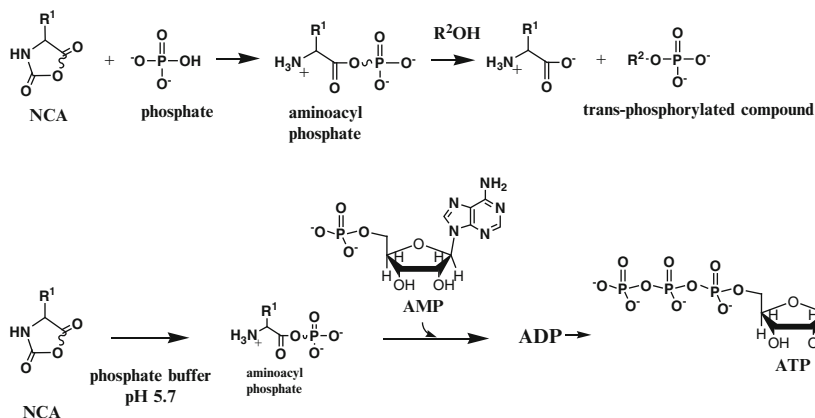

Fig. 6.9 Alcohols as AMP or ADP can be phosphorylated by aminoacyl phosphate

the presence of the enzymatic Luciferin/luciferase system (A. Commeyras, L. Garrelly, H. Collet unpublished data).

In this chapter, and by extrapolation, we have assumed that the different nucleoside triphosphates (NTP) have been obtained by the same reaction. This reaction shows that the primary pump may share its energy with phosphates. This property illustrates the vision of prebiotic reactions by Prof. Albert Eschenmoser (Eschenmoser 2007), that is to say:

> The less "robust" (constitutionally unidirectional) chemical reactions in a given environment are, the more sensitive and responsive to catalytic acceleration and inhibition, and the higher will be their chances to become assisted and eventually steered by catalysts that may contingently be present or be emerging in the environment.

In this scenario, the primary pump is a special device that continuously produces a family of energetic molecules, the NCAs. A fraction of these NCAs are used to form peptides. Others react with phosphates, which are distributed in many directions. We illustrate below how a possible pathway for the generation of nucleic acids could have emerged.

6.4.3   Concentration and Stabilization of Nucleoside Triphosphate (NTP)

6.4.3.1   Dendrigraft α-Amino Acids

In developing other goals of using the primary pump concept, we have shown (Collet et al. 2010) that it is possible to produce dendrigraft lysine (DGL), polymerizing lysine NCA in water at pH between 5 and 7, that is to say in potentially prebiotic conditions. Successive generations of DGL are obtained using as an initiator for the generation (n) the product of the generation ($n - 1$). The molar mass of these dendrigraft lysines grows exponentially. Table 6.1 gives some characteristics of DGL through five generations.

Figure 6.10 idealizes the structure of DGL-G3. Such a structure is a nano-sphere of 7 nm in diameter fully soluble in water. The amine functional groups of these nano-particles are localized at the surface of the nano-sphere. In water at pH 5–6, the amine functional groups are fully protonated, DGL particles are polycationic. Under prebiotic conditions, of course, other amino acids would be interspersed stochastically and the degree of branching would be less compact.

On the beaches of the primitive continents, such dendrigraft amino acids (DGA) could have been produced in a few weeks through wet and dry cycles. If this was the case, obviously strong interactions have existed between these polycationic DGA, and NTP, which under the same conditions are polyanionic (3 negative charges per NTP molecule).

6.4.3.2   Concentration of NTP at the Surface of DendriGraft Amino Acids (DGA)

The polycation–polyanion interactions have been studied in the case of DGL-G3 and ATP.

It is shown that the affinity constant between DGL-G3 and ATP is equal to 10^6 M^{-1} at pH 7.4 at 8 mM ionic strength (Zou et al. 2010).

The complex between these two molecules is composed of 25 ATP per molecule of DGL-G3.

Assuming that ATP is mainly localized to the surface of DGL, the structure of the complex can be visualized by Fig. 6.11.

If the ATP molecule has a thickness of 1 nm, the local ATP concentration can range from 0.2 to 0.3 moles per liter. These high values are largely beyond the minimum required.

Table 6.1 Some characteristics of dendrigraft lysines of generations 1–5

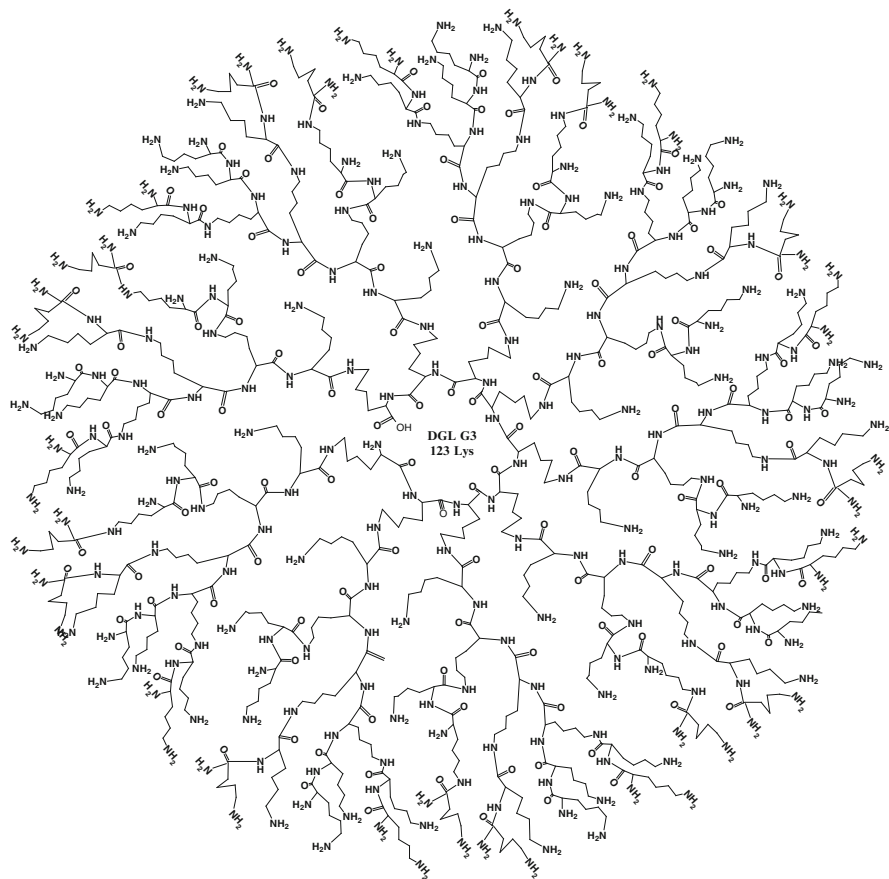| Generation	1	2	3	4	5
M_n (g/mol)	1,450	8,600	22,000	65,300	172,300
N (number of lysines)	8	48	123	365	963
R_h (nm) at pH 7	1	2	3.5	4.5	6

Fig. 6.10 Idealized structure of DGL-G3

6.4.3.3 Stabilization of ATP (NTP) in the Complex DGL-G3/ATP

By measuring the rate of hydrolysis of ATP in the absence and presence of DGL, we observed that the presence of DGL significantly slowed the hydrolysis of ATP so that at temperatures of 30 °C or below, ATP is stable for months. This stability in the complex DGL-ATP does not prevent the ATP to be active in the presence of a modern enzyme system. We have indeed shown that it is possible to determine the total ATP content in the complex DGL-ATP using the reaction of ATP on the enzymatic system Luciferase/Luciferin. (A. Kovalova, L. Garrelly, A. Commeyras unpublished data). This accessibility of ATP to enzyme systems may have been of evolutionary importance.

This result can certainly be extended to any kind of complex NTP/polycations. We ought, however, not to forget that in living organisms, the NTP and beyond nucleic acids are mainly associated with peptides. The study of interactions polyanions/polycations is difficult but of great interest.
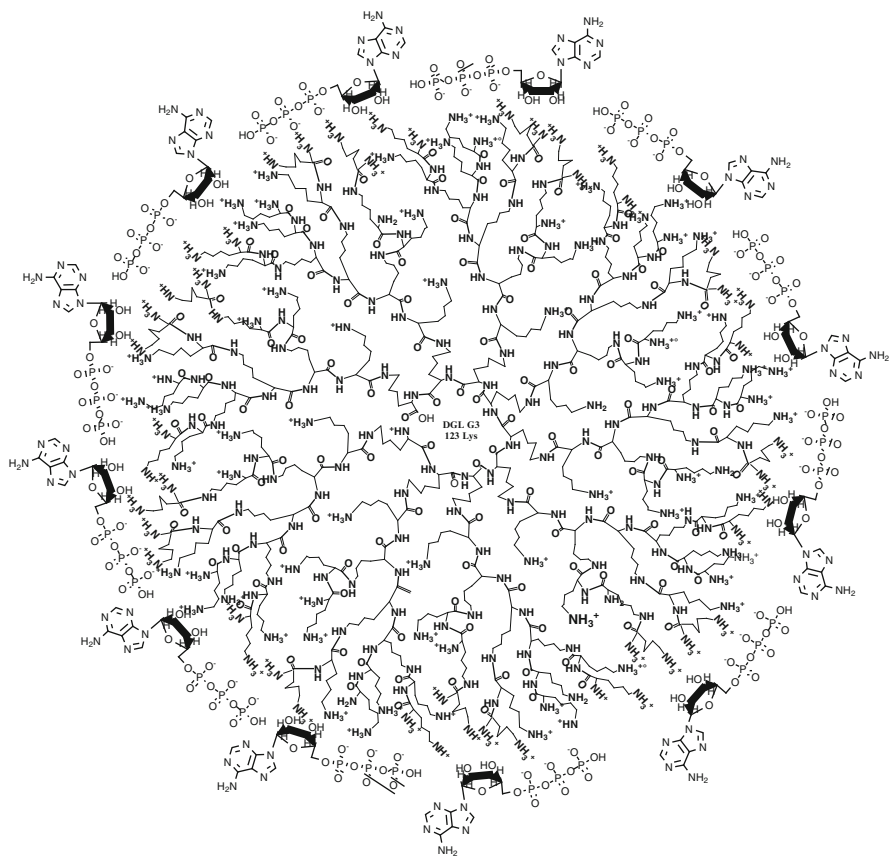
Fig. 6.11 Idealized complex DGL-G3/ATP. Interactions polyanions/polycations concentrate and stabilize the peptides on dendritic NTPs. These could serve as a template to promote the condensation of the NTPs.for the polymerization of activated nucleotides. In the scenario of the primary pump the NTPs are formed every day but certainly at very low concentrations. In diluted solutions they can hydrolyse, or be concentrated in the complex DGA/NTP. – The question is then to know if NTPs must have been stabilized or not in the complex DGA/NTP

6.4.4   The Primary Pump and Nucleic Acid Synthesis

No experiment has yet been done on whether the NTP concentrated and stored in the complex DGL-NTP can be condensed. The following is therefore purely speculative.

One can only wonder:

- Whether this condensation is possible or not?
- If it is possible, does it take place in the aqueous phase, or rather in dry phase only?
- Whether the pH played a role in condensation?

- Whether a catalyst was needed or not?
- If catalysis was needed, were catalysts present beforehand or later-emerging in the environment?

Indeed we have seen, in the scenario of the primary pump, that the primary structure of peptides is constantly changing. It is also known that catalytic activities are present in non-coded dendrigraft amino acids (Darbre and Reymond 2006). Therefore, it is reasonable to assume that catalytic activities able to promote the polymerization of NTP may have emerged from such environments.

And to go beyond, if nucleic acids are formed, they will hydrolyse slowly, and a new cycle reaction will appear. This cycle will be focused on nucleic acids synthesis, with activation, condensation of nucleotides, partial hydrolysis of nucleic acids, new activation, and so forth. The template of this new cycle will always consist of dendrigraft peptides that will gradually become homochiral. These templates will select chiral nucleic acids, and so on.

This new cycle will work through the constant supply of energy provided by the primary pump – comprising the heart of the primordial evolutionary engine.

6.5   In Brief

1. The primary pump is a driving force of a global system.
2. Every day, the primary pump built the NCA from which dendrigraft amino acids are formed.
3. The structure of these peptides are evolving.
4. Via trial and error the most likely molecules are subject to chemo- and enantio-selection.
5. The chemical reactions are assisted by catalysts present, or potentially emerging in the environment
6. Concentration processes used are simple and natural.
7. In the scenario of the primary pump, reaction pathways are short. Matter and time are saved. The key molecules are recycled.
8. The primary pump shares its energy with phosphoric acid.
9. This distribution of energy leads to a permanent activation of nucleotides into nucleotide triphosphate (NTP).
10. The primary pump, via dendrigraft peptides, concentrates and stabilizes NTP.
11. NTP concentrated and stabilized in a complex of Dendrigraft Amino acids (DGA)-NTP may have led to nucleic acids.

The scenario of the primary pump appears to be co-evolutionary. This means that a chemical energetic system pushes the peptides and potentially nucleic acids to co-emerge and co-evolve in the same environment.

6.6   How Long has the Primary Pump Worked, and What Conditions Stopped its Operation?

The primary pump may have worked as long as the proportion of NO in the mixture NO/O_2 remained high (>4). This could correspond to the initial conditions of the primitive Earth in which oxygen was scarce. When the proportion of oxygen increased, the concentration of nitrosating agents decreased dramatically and the pump gradually stopped working.

Primitive life has then adapted to the increased concentration of oxygen when the role of NO decreased. But the role of NO may not have completely disappeared, since in modern life NO is a neurotransmitter of all living organisms. One wonders whether it would be a fossil remnant of early times or it would have played a role in the complexification of the organic matter. This potentiality could be an additional element to our thinking.

6.7   General Conclusion

The primary pump scenario is a tool (like the "intuition pump" according to Daniel Dennett) that allows us to understand the transition from inert to living matter. By continuous pumping, the primary pump could have led to something new and significant.

This should be satisfying to the unstoppable reasonist Shadok (French Cartoon) in his relentless determination to continue pumping (Fig. 6.12), "It is better to pump even if nothing happens, rather than risk something worse happening if we do not pump."

Fig. 6.12 Shadok's motto: *"It is better to pump even if nothing happens, rather than risk something worse happening if we do not pump"*

«Devises Shadok – Il vaut mieux pomper même s'il ne se passe rien que risquer qu'il se passe quelque chose de pire en ne pompant pas.» (Jacques Rouxel)

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