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From prebiotic chemistry to cellular metabolism—The chemical evolution of metabolism before Darwinian natural selection ☆

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Abstract

It is generally assumed that the complex map of metabolism is a result of natural selection working at the molecular level. However, natural selection can only work on entities that have three basic features: information, metabolism and membrane. Metabolism must include the capability of producing all cellular structures, as well as energy (ATP), from external sources; information must be established on a material that allows its perpetuity, in order to safeguard the goals achieved; and membranes must be able to preserve the internal material, determining a selective exchange with external material in order to ensure that both metabolism and information can be individualized. It is not difficult to understand that protocellular entities that boast these three qualities can evolve through natural selection. The problem is rather to explain the origin of such features under conditions where natural selection could not work. In the present work we propose that these protocells could be built by chemical evolution, starting from the prebiotic primordial soup, by means of *chemical selection*. This consists of selective increases of the rates of certain specific reactions because of the kinetic or thermodynamic features of the process, such as stoichiometric catalysis or autocatalysis, cooperativity and others, thereby promoting their prevalence among the whole set of chemical possibilities. Our results show that all chemical processes necessary for yielding the basic materials that natural selection needs to work may be achieved through chemical selection, thus suggesting a way for life to begin.

Keywords: Origin of life; Evolution of metabolism; Chemical evolution; Prebiotic chemistry

1. Introduction

Professor Reinhart Heinrich was always keenly interested in biological evolution and natural selection, and especially in the evolution of metabolism. His first works in this field dealt with the optimization of the kinetic parameters of enzymes to produce maximum activity (Heinrich et al., 1987; Heinrich and Hoffmann, 1991), a subject that had been previously explored by Cornish-Bowden (1976). Over the course of time, our mutual scientific interest fomented a close friendship as well as a fruitful scientific collaboration. We had many meetings in Berlin, Tenerife, and Madrid, continually discussing evolution, metabolism, and optimization. Fruit of these discussions was a series of papers on glycolysis optimization (Heinrich et al., 1997a, b, 1999; Meléndez-Hevia et al., 1997; Waddell et al., 1997, 1999) and one which presented his difficult theory on optimization of enzyme parameters in such a way as to make it more accessible to students (Heinrich et al., 2002).

In the last years, as of 2003, we had initiated a very ambitious task: the search for the history of the appearance of all metabolic pathways. In some interesting discussions, Heinrich said that by looking at the structure of a city, it could be possible to know the history of its origin (for example, whether it had been built by radial growth from a small nucleus, like Madrid, or by joining small villages, like Berlin), and that perhaps the historical development of

☆This paper is dedicated to the memory of Professor Reinhart Heinrich, who was collaborating with us in this subject, and died suddenly when this work was almost finished. His name should be as co-author but it has not been possible, because, with great sadness, we have been told that we had to respect his criterion that he firmly stated of not signing any paper without having seen the last version.

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metabolism could be unveiled in the same way. This observation opened up new modes of inquiry for us and we were soon rewarded with some clear insights. For example, the Calvin cycle uses ribulose 5-P as feeder, so this product had to be available beforehand, and because ribulose 5-P is made only by the pentose-phosphate cycle, we concluded that the emergence of this pathway had to be prior to the Calvin cycle.

Heinrich had another great idea: he though that if we were able to uncover the history of metabolic pathways, it would be possible to calculate the stoichiometric matrix of each stage in its evolution, which would then allow us to have quantitative information about the progression of metabolic complexity. Heinrich designed a computer program to that end and we began our quest.

Heinrich wanted to plot the progress of metabolic complexity over time from its origins, and we needed to explore its history through a logical retrospective reasoning going back to the beginning of metabolism, the end of which would be the origin of life. In sum: the stoichiometric analysis could not be undertaken until its history was accounted for in full. Heinrich started to make calculations with the first results, but they could only be provisional until all metabolic history was completed. We were nearing the end of our task when we learned of the tragic news of Heinrich's untimely death. It is to his memory that we would like to dedicate this paper, both because he participated in this research, and because his ingenious ideas continue to inspire us.

Darwinian natural selection is a powerful mechanism capable of developing many optimized structures and functions, as well as of creating new ones, based on their selective value under competitive conditions This has been specifically studied at the molecular level by Baldwin and Krebs (1981), Cornish-Bowden (1976), Heinrich and Hoffmann (1991), Heinrich et al. (1987, 1991, 1997a, b, 1999), Kacser and Beeby (1984), Meléndez et al. (1997), Meléndez-Hevia and Isidoro (1985), Meléndez-Hevia and Torres (1988), and Meléndez-Hevia et al. (1993, 1994, 1996, 1997).

However, if we try to explain the origin of life by natural selection, a critical problem appears: natural selection can only work if it has three basic materials: information, metabolism and membrane. Therefore, at the origin of life, these had to be achieved previously for natural selection to work, and these qualities could only be done by a prior process of pure chemistry in which any feature of interest for life had no special importance. Therefore, a purely chemical process had to occur prior to natural selection capable of yielding these materials specifically, independent of their value for building life later. In this work we have explored the chemical possibilities of prebiotic chemistry to produce these materials and conclude that prior to natural selection, a previous process only governed by the rules of chemistry must have occurred in order to yield the appropriate materials. Moreover, we show that such a process could be highly favoured, as the chemical reactions involved in it are autocatalytic and/or cooperative.

2. Hypotheses and rules

2.1. General hypotheses for evolution of metabolism

In a previous work (Meléndez-Hevia et al., 1996) four rules of metabolic evolution were stated as follows:

2.1.1. Bioorganic chemistry

1. Any enzymatic reaction is also chemically possible without the enzyme, although in that case it would occur much more slowly and without a well-defined specificity.
2. All the intermediates of a chain of reactions to be used ultimately in a metabolic sequence must resist rapid decomposition. The strongest reason for this assumption is evolutionary; at the beginning of the pathway design every rudimentary enzymatic reaction occurred very slowly, so unstable intermediates could not have been used.
3. *Material availability* (*Opportunism*): Any material to be used by the new pathway must exist in another metabolic process which was originally developed for a different purpose. Design of this new pathway must preserve the function of the previous one whose material has been used. An inverse chronological application of this rule would eventually lead to the origin of metabolism; on the primordial Earth the first available compounds had to have been made through spontaneous chemical processes.
4. *Kinetic and thermodynamic compatibility*: The new pathway cannot have a reaction involving any thermodynamic or kinetic incompatibility with a previous one that is operating simultaneously in the same space.

2.2. Specific hypotheses for the origin of life

The rules mentioned above are the first basic hypotheses of this work. In addition, we shall add others specifically stated for our approach to search for the sequence of events in time that have developed our present metabolism from prebiotic chemistry.

1. *Universality of intermediary metabolism*: The universality of intermediary metabolism has been remarked on by several authors (Morowitz et al., 2000; Smith and Morowitz, 2004), assuming that the basic structure of intermediary metabolism represented by the classical wall chart (see Michal, 1999) is universal. Although there is a general consensus on this point, different parts in the metabolic map must nevertheless be distinguished: on one hand, the central pathway of carbon flux, which is universal, with few exceptions, and on the other hand, the pathways to make ATP, and the pathways to fix carbon and nitrogen, where there is a broad diversity. This distinction is interesting for the purpose of this work.

2. *Continuity of metabolism*: This hypothesis is the same rule of the continuity of life. We assume that the early organization of metabolism has evolved to its present-day form by means of small changes, as stated by Darwin, without modifying its basic structure. In modern terms, evolution is a continuous function, in which all intermediate stages are useful. This condition has been considered by several authors in models on metabolic evolution (see Cairns-Smith and Walker, 1974; Cairns-Smith, 1982; Meléndez et al., 1997). Many important changes—such as the appearance of eukaryotic cells, or the divergence between animals and plants—have occurred in the course of evolution. However, such events have not modified the general chemical sequence of reactions in the pathways stated above. Thus, the denial of this hypothesis would lead to creationism, involving several independent emergences of life, in the same way that in XIX century Cuvier and others tried to explain the first uneven data of the fossil record by a series of many great catastrophes like floods which periodically wiped out all life, interspersed by new creations (see a discussion of this in Meléndez, 1998).
3. *Prebiotic conditions—the primordial soup*: Prebiotic conditions include alkaline pH, high hydrogen, ammonia, and CO_2 levels in the atmosphere, as well as formaldehyde and hydrogen sulphide (H_2S), and some other minerals, such as phosphate, pyrite, and soluble salts, which intervene in the present metabolism at different points. These conditions have been extensively described and enjoy a general consensus (see Miller, 1953; Maden, 1995).

The objective of the present work is to propose a biochemically sound continuity between primitive chemistry and modern life. That this is possible is the subject of our discussion below. Here, we present a plausible model that can explain the emergence of that map based only on the available material, respecting all mechanistic rules of chemistry and the hypotheses stated above.

3. Approach

3.1. Retrospective analysis

The set of hypotheses stated above allows us to carry out a logical retrospective reasoning going back to the beginning of metabolism, by means of a successive inverse chronological analysis until prebiotic chemistry. By applying to the present scheme the hypotheses of *opportunism* (the pathways are connected through their starting points on a main one, following a time of emergence sequence), and *continuity* (metabolic interactions have persisted in time) we can reconstruct many segments of the history of metabolism. We assume that metabolism started from the primordial soup, and we present here a scenario demonstrating that the emergence of the minimal necessary metabolic map is possible from prebiotic chemistry.

3.2. Chemical mechanisms of metabolic reactions

Every metabolic reaction implies two different chemical mechanisms: the '*stoichiometric*' mechanism, which is the real reaction mechanism accounting for the chemical transformation of the reagents, and the *catalytic* mechanism, which accounts for the reaction rate increase. The second is built on the first by enhancing the reactivity of the substrates and stabilizing the transition complexes, but does not modify it, as the stoichiometric mechanism is independent of the catalytic one. It is interesting to see that in some cases, the same stoichiometric mechanism can be performed by two different catalytic mechanisms. This occurs, for instance, in type I aldolases, which are catalyzed by a lysine residue of the enzyme, and type II aldolases which are catalyzed by zinc (Rutter, 1964; Baldwin et al., 1978) and in hydrolysis reactions, where the same stoichiometric mechanism (a nucleophylic attack of HO^-) is achieved by a serine residue in serine proteinases (see Wharton, 1998), or by H_2O in aspartic proteinases (see Meek, 1998). This feature is stated in Hypothesis 1.

Our model is based on reactions that exist in present-day metabolism, catalyzed by enzymes which actually exist, in order to explain the continuity of metabolism. However, our approach is not based on the existence of these enzymes as catalysts, but on the chemical possibilities that make these reactions possible.

3.3. Coenzymes

Metabolic reactions can be explained without enzymes by their stoichiometric chemical mechanisms, as stated above, but coenzymes are necessary because they participate in the chemical stoichiometric mechanisms. Thus, the emergence of metabolism must also explain the appearance of coenzymes. This question might seem that leads us to an apparent problem with no solution, as most coenzymes intervene in the pathways of their own synthesis, which looks like the old riddle of the chicken and the egg. On the other hand, coenzymes are very complex molecules whose origin from prebiotic chemistry is hard to explain. However, most of the chemical structure of the present coenzymes is only necessary for the specific interactions with the enzymes, but not for reaction mechanisms (see Sinnott, 1998). For instance, only the nicotinamide group is necessary in $NAD(P)^+$, the thiazol ring in thiamine, pyrophosphate in ATP, etc. Thus, we can assume that the coenzymes that participated in early metabolism must not have been as complex as the current ones. The only requisite for explaining the emergence of the metabolism we are discussing here is to find a chemical explanation for present-day enzymatic reactions. In this way, all current complex coenzymes could be substituted by very simple mineral compounds. For example, CoA could be substituted by a sulphur compound, such as sulphite, because the only requirement for making the acyl-CoA reactions

possible is the sulphur atom (which can break down the resonance of the carboxyl group); thiamine could be substituted by any chemical cation such as Zn^{2+}, capable of stabilizing a carbanion group; tetrahydrofolate (THF) could be substituted by a chemical reagent, such as sulphide or sulphite, capable to stabilize a carbacation group; NAD(P)H can be substituted by a metal hydride, such as $NaAlH_4$; metal hydrides do not occur at present in the Earth, due to the oxidative environment, but they were actually possible under a reductive environment; etc. In effect, it has been demonstrated that some inorganic compounds can function as metabolic coenzymes under prebiotic conditions (Keefe et al., 1995), and indeed, some vestiges of this remain in modern metabolism, like Darwin's lost rings, which we have called *paleometabolism* (Meléndez-Hevia et al., 1994); examples of this can be pyrophosphate working in glycolysis instead of ATP (Mertens, 1993), or Zn^{2+} instead of the lysine residue stabilizing the carbanion group in class II aldolases (Baldwin et al., 1978). Thus, since most of the structure of coenzymes does not participate in the reaction mechanism, but in its affinity with the enzymes, it suggests that the present coenzymes appeared through co-evolution with protein enzymes in later stages of metabolism development. Nonetheless, in this paper we shall, for the sake of brevity, refer to the coenzymes as they exist at present, i.e., ATP, NAD^+, $NADP^+$, THF, etc.

4. Results

4.1. General considerations

4.1.1. Requisites for natural selection

The minimal material for Darwinian natural selection is information, metabolism and membrane. Replicative information is obviously necessary, in order to consolidate the goals attained, allowing for further growth and development. Metabolism involves both the production of energy and the chemical conversion of external material into internal structures, including the building of the new informative material and membranes, as well as the products that natural selection needs to work. Finally, membrane is necessary because natural selection can only work if the system becomes selfish, i.e., when its information is individualized. This minimal material for natural selection is also the minimal material for life, as both are one and the same.

Among the different models proposed to explain the origin of life, the metabolism-repair systems, or *MR systems* (see Rosen, 1991) deserve special attention, as abstractions for defining the minimal possible metabolism for the emergence of life. The central idea of this model is that the first metabolic systems had to be able to produce their own material from environmental sources, which implies a semi-circular work. However, as Cornish-Bowden and Cárdenas (2008) have pointed out in a paper in this issue, a major feature of such MR systems must be their metabolic individuality, versus other systems, in order for natural selection to operate. This implies membrane and perpetuated information, whose materials must also be built by this metabolism.

It is clear that the *catalytic mechanisms* carried out by macromolecules (RNA or proteins), are products of natural selection, as several authors have pointed out; see, e.g., Cornish-Bowden (1976) and Kacser and Beeby (1984). This can explain the fairly catalytic diversity working on the same chemical stoichiometric mechanism that we find in the present life, which again tells us that the stoichiometric mechanisms existed first. Thus, the main problem of the pre-enzymatic chemical evolution is to explain how chemistry itself could account for the selection of metabolic reactions previously to enzymatic catalysis, which is the central aim of this work.

4.1.2. Chemical selection

The prebiotic synthesis of some compounds that exist in the present metabolism has been demonstrated. These include several amino acids (Miller, 1953, 1995), adenine and other purine bases (Oró, 1965; Oró and Kimball, 1961) and a number of sugars (Mizuno and Weiss, 1974; Reid and Orgel, 1967), among others; see a review in Orgel (2004). The prebiotic synthesis of these products might be carried out by the same or different routes as the present-day pathways. For instance, the prebiotic pathway for purine synthesis could be the same as that of the current metabolism, as we discuss below, while the present photosynthetic pathways to synthesize glucose from CO_2 are obviously different from the formose prebiotic reaction. In any case, prebiotic chemistry had to evolve to yield the present metabolic map.

On the other hand, a chemical pre-enzymatic selection was necessary because a small number of reactions or pathways had to be selected among the huge set of possibilities of prebiotic chemistry, in order to lead to present-day metabolism. It is possible that some specific catalytic mechanism existed, which were able to select certain prebiotic reactions, as has been suggested by various authors (Cairns-Smith, 1982; Weber and Pizzarello, 2006). However, these mechanisms cannot obviously explain the emergence of the metabolic map in full, though the possibility of a magnification of certain reactions by kinetic or thermodynamic advantages could facilitate its development. Thus, the history of metabolism must explain that this process is both chemically possible, and that it is favoured over other chemical possibilities. Natural selection came later, magnifying sets of reactions (pathways) to give products with a clear biologically selective value.

Life evolved through Darwinian natural selection, and its earliest form appeared when natural selection became possible. Thus, the appearance of the minimal material necessary for natural selection to work could not be produced by natural selection itself, but by a previous selection that we shall call 'chemical selection'. This consists

of an increase and enlargement of certain specific chemical processes based on specific kinetic and thermodynamic features that can increase the reaction rate. Yet without considering the existence of enzymes as catalysts specific for particular reactions, there are a number of different mechanisms that can enhance the reaction rates based only on the *stoichiometry* of the pathways, i.e., without 'external' catalysts: (a) stoichiometric catalysis, which occurs in all metabolic cycles, as the feeder (or starter) does as a catalyst itself; (b) stoichiometric autocatalysis, which occurs in some cycles when the global reaction yields more amount of the feeder than its entrance, so promoting the enlargement of the reaction (see examples below); (c) thermodynamic cooperativity, which is a property of a process in which physical or chemical positive interactions among the end products enhance the stability of the final structure promoting their production. This effect is typical in the construction of polymers, as these processes imply an initiation step, which is usually difficult, as it is delayed by a positive energy change, followed by another of elongation that is much easier; and (d) thermodynamic push of certain specific processes by products originated in others; e.g., ATP production will promote biosynthetic reactions, and NADPH production will promote reductive processes.

4.2. Specific points—sequence of events

4.2.1. The metabolic map

The metabolic map of the carbon flux (see, e.g., Michal, 1999) looks like a very intricate labyrinth with plenty of connections among enzymes and intermediates where the deciphering of any chronological hierarchy among them could seem rather difficult. However, that task is not impossible. First we can define 'metabolic pathways' as chains of reactions that connect an existing initial substrate, available from an external source (food) or from an intermediate of another pathway, starting from it as a branch, with an end product, which has a specific global or partial purpose. If we consider only the biosynthetic pathways that lead to the products necessary for the minimal metabolism pointed out above, then, the complex metabolic map, reduced to only the most essential metabolism, becomes very simplified and can be draw as the scheme shown in Fig. 1. This is clearly a central skeleton formed by glycolysis, starting from glucose as the general source of organic carbon material, and by the horseshoe Krebs 'cycle', from which all other biosynthetic pathways start as branches from this central structure. Application of the retrospective analysis principle to this scheme suggests that glycolysis was a first central pathway

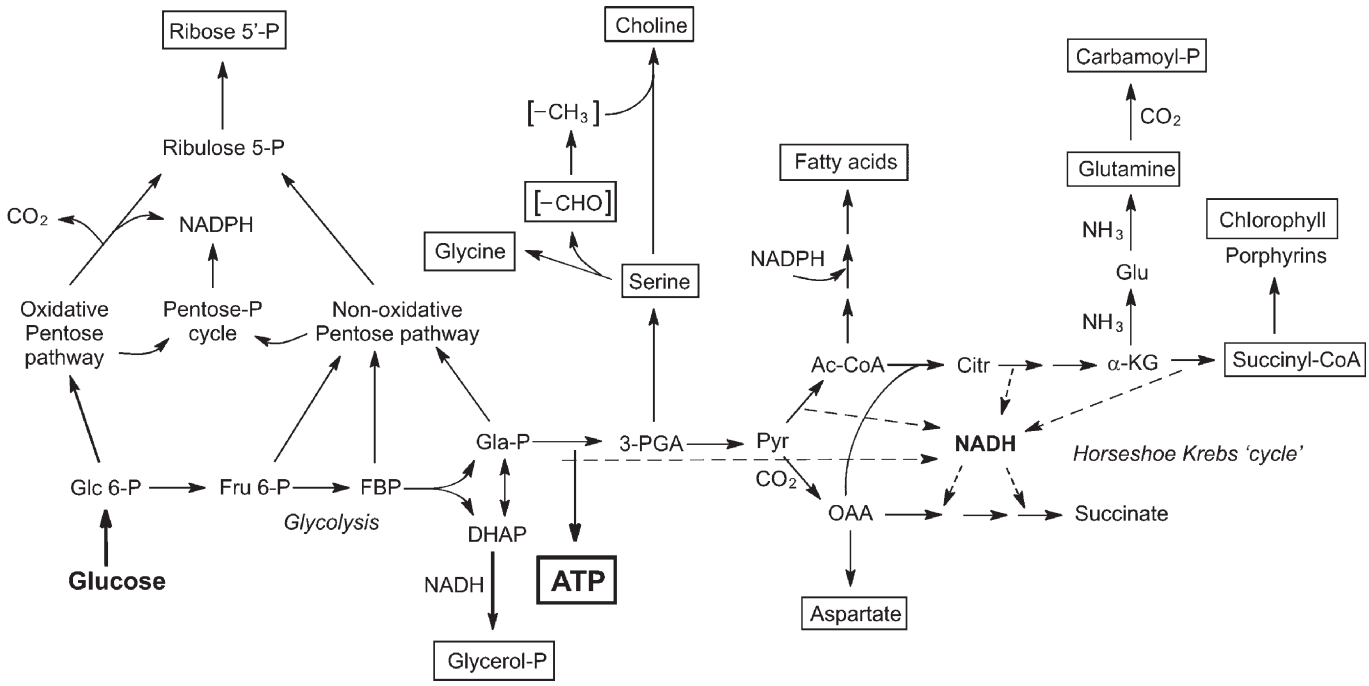

Fig. 1. Minimal metabolic map made by simplifying present-day cellular metabolism that includes the synthesis of all products necessary for making the basic materials that natural selection needs to work: information, metabolism and membranes. Purine nucleotides are made from ribose, glutamine, glycine, formate, and aspartate. Pyrimidine nucleotides are made from aspartate and carbamoyl-P; membrane phospholipids are made from fatty acids, glycerol-P and choline or serine. The system starts from glucose that is taken from the primordial soup where it has been synthesized by the formose reaction. The redox state for the pair NAD^+/NADH (its constant ratio) is compensated in the horseshoe Krebs 'cycle', or producing glycerol-P. NADPH produced by the oxidative pentose-phosphate pathway can be consumed by fatty acid synthesis and other biosynthetic processes, such as glutamate synthesis. The map clearly suggests that all cellular metabolism was built from glucose, through glycolysis and the horseshoe Krebs 'cycle', forming the central frame from which all other pathways emerged.

built in the history of present-day metabolism, which could start from glucose.

The development of metabolism had to start from prebiotic chemistry, but its critical goal was to become independent from it, achieving mechanisms capable of generating organic material from the mineral world (CO_2, H_2O and NH_3, mainly) as well as producing an energy currency (ATP), necessary for biosynthetic processes. As we have pointed out above, the energy currency could be simply pyrophosphate, which is the essential reactive group of ATP. It is important to notice that the production of an energy currency not only allows the development of new pathways, but also *favours* them thermodynamically and even kinetically, because ATP intervenes as coenzyme supplying a suitable reaction mechanism, which determines the emergence and prevalence of biosynthetic pathways. Thus, this provides a good argument for considering the first metabolism to be biosynthetic.

4.2.2. *The formose reaction*

Condensation of formaldehyde under mildly alkaline conditions, known as the formose reaction, produces a wide variety of sugars. This reaction was discovered by Butlerow (1861), and later studied in detail by Mizuno and Weiss (1974), and other authors (see the chemical mechanisms in Fig. 2). The experiments carried out on the formose reaction showed that, among of all sugars produced, glucose is the most abundant product (see Cairns-Smith and Walker, 1974; Mason, 1992), which understood as β-cyclic D-glucose is the most stable sugar

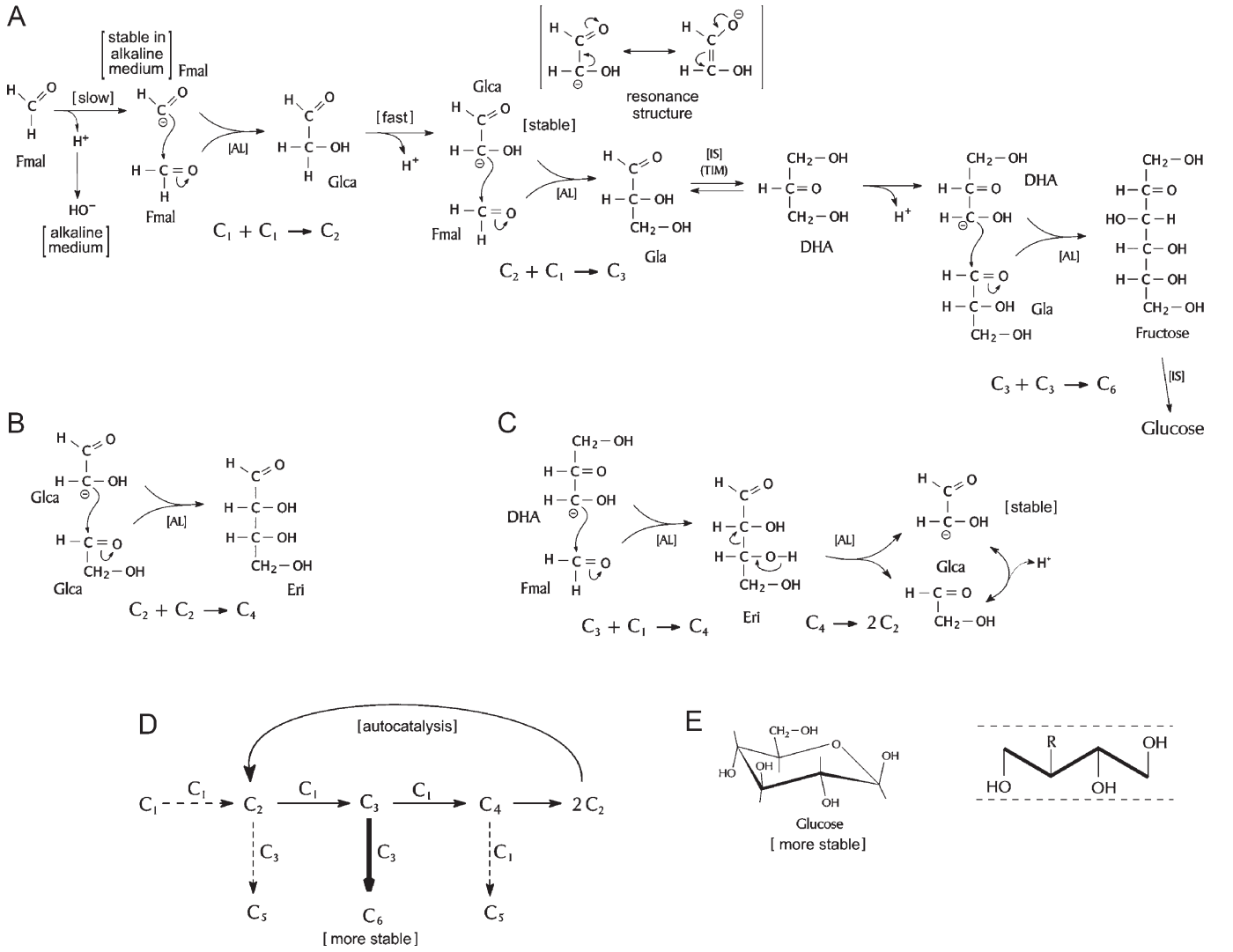

Fig. 2. Chemical mechanisms of the formose reaction: (A) sequence of reactions from formaldehyde to glucose by three formaldehyde condensations to the triose-phosphate followed by the condensation between two trioses to produce hexoses, as occurs in modern glycolysis; (B) condensation between two molecules of glycolaldehyde to produce a tetrose; (C) a different possible mechanism to yield a tetrose by condensation between glyceraldehyde and formaldehyde, and the cleavage of the tetrose to yield glycolaldehyde, which is the most reactive substrate. Different combinations of these reactions can yield a number of different sugars, as well as autocatalytic mechanisms; (D) an example of an autocatalytic pathway based on glycolaldehyde; and (E) picture showing the stability of the glucose molecule, as its cyclic structure is an hexagon ring with all substituting groups in equatorial position. *Abbreviations*: DHA, dihydroxyacetone; Eri, eritrose; Fmal, formaldehyde; Gla, glyceraldehyde; Glca, glycolaldehyde.

because of its hexagonal structure, all external groups being in equatorial position (see Fig. 2E).

It must be noticed that an alkaline environment promotes reductive conditions, under hydrogen atmosphere, via $H_2 \rightarrow H:^- + H^+$, as an alkaline pH is a sink of protons directing the reaction to hydride formation. Under hydrogen atmosphere, the extent of the alkaline conditions determines the level of reductive power. Thus, as it is assumed that this reaction could occur under prebiotic conditions (see Mason, 1992; Orgel, 2004), it makes the former scenario more plausible because it can explain the origin of glycolysis as a main support of central metabolism. Several authors have claimed that the formose reaction could be not important in the origin of metabolism, because glucose has not been found to be a main product in prebiotic experiments (see a discussion on this subject in Orgel, 2004). This must be due to the fact that the reaction is very slow at the beginning (see Fig. 2A). However, the autocatalytic nature of the formose reaction, shown in Fig. 2D, as it has been remarked upon by several authors (Mason, 1992; Orgel, 2004), strongly suggests that it could play an important role in the origin of metabolism.

4.2.3. Glycolysis

Glycolysis has several interesting features that make it a very appropriate pathway for starting metabolism: it accounts for the supply of many products useful for the building of other pathways and, moreover, it yields the general energy currency (ATP). On the other hand, glycolysis is a very versatile pathway as it is capable of adopting many different designs that can be coupled with other processes to yield material, ATP or both, and it can also be adapted to different environmental conditions, such as an ATP source under aerobic or anaerobic conditions. It can work as a source of glycerol to make lipids, or as a source of ribose, through the pentose-phosphate pathways, to produce the informative material, and oxaloacetate and acetyl-CoA, which are the starting points of the horseshoe Krebs cycle. On the other hand, as an ATP source, glycolysis will promote the development of biosynthetic pathways, which together with other ATP sources are facts that can explain the routing of this premetabolic chemistry in specific biosynthetic directions, which is one of the features of chemical selection.

Indeed, it must be taken into account that although we derive this conclusion concerning glycolysis as the initial pathway of the central metabolic structure, and the origin of virtually all metabolism, from the simplified map shown in Fig. 1, this statement has no loss of generality, as the same conclusion can be derived from the full metabolic map. It must be noticed that the whole set of glycolysis plus the horseshoe Krebs cycle is redox independent, as it does not depend on an external source or sink of electrons (Meléndez-Hevia et al., 1996). Considering the present structure of the pathways shown in Fig. 1, a retrospective analysis by applying the hypotheses of opportunism and continuity yields the results shown in Fig. 3.

It is possible, however, that other specific pathways could have been formed under prebiotic conditions. Purine biosynthesis (Oró, 1965; Oró and Kimball, 1961) and glycine (Miller, 1953, 1995) can be examples of pathways originated in prebiotic chemistry and later incorporated to the central metabolism. It could be considered, in fact, that some of these pathways existed before glycolysis. However, none of them support a retrospective analysis as being the central pathway for building the present metabolic map. It is clear, looking at Fig. 1 that the metabolic map has not been defined on any of these other pathways as its central skeleton, as all of them are specific branches for yielding particular compounds. These pathways could be developed at the same time of glycolysis, or even earlier, but they had to be adapted to glycolytic products to guarantee their continuity.

It can be considered that the primordial soup also contained other organic compounds, such as formic acid, glycine, aspartate, carbamate, purine bases, etc., whose synthesis has been experimentally demonstrated by several authors under prebiotic conditions (Miller, 1953, 1995; Oró, 1965; Oró and Kimball, 1961). However, our analysis shows that these products were not necessarily starting points for the metabolic pathways, but rather intermediate confluence points which could serve as backup for the development of a definitive metabolism favouring the emergence of the metabolic map.

4.2.4. Krebs' cycle

A metabolic cycle is a pathway with two different entrances: the substrate of the global conversion (e.g., acetyl-CoA in Krebs cycle, or carbamoyl-phosphate in the urea cycle), and the feeder, which is not globally transformed (e.g., oxaloacetate in Krebs' cycle, or ornithine in the urea cycle). Cyclic pathways have a feature that awards them a clear kinetic advantage for chemical selection: they are catalytic, because the feeder plays a role similar to a catalyst (see Baldwin and Krebs, 1981).

The opportunism hypothesis states that in addition to the substrate, a metabolic cycle can only be built if the feeder exists previously because it is involved in another metabolic process that was built for a different purpose. Thus, the Krebs cycle, e.g., as a way to use acetyl-CoA, was obviously built after acetyl-CoA and oxaloacetate, both compounds having previous different metabolic purposes.

Thus, the trouble with explaining the origin of the Krebs cycle as a catabolic pathway is in justifying the previous presence of oxaloacetate. However, if the Krebs cycle was built from the horseshoe design, as a biosynthetic pathway, then, since it is a double linear (non cyclic) pathway, it does not need to explain previous purposes, as acetyl-CoA is the precursor of glutamate and succinyl-CoA (which is the precursor of the heme group), while oxaloacetate, as the precursor of aspartic acid, can be the substrate of the other branch whose purpose is to maintain the anaerobic conditions. The final cyclic design of this pathway, to be

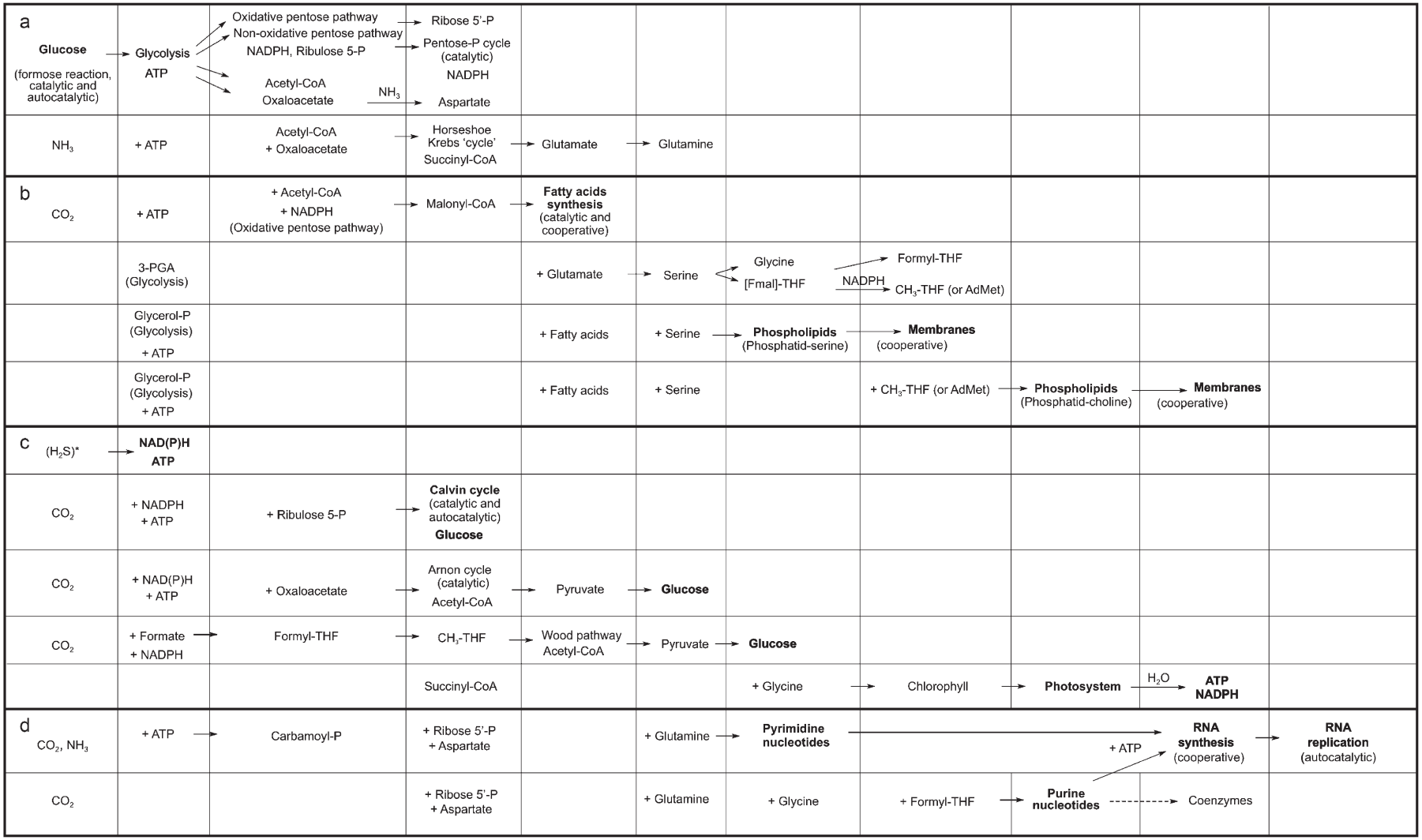

Fig. 3. Biosynthetic pathways that natural selection needs to work, only considering reactions that exist in present-day metabolism. Columns represent the steps of the processes, and rows represent the pathways. The first column (left) contains the precursors present in the primordial soup and the primitive atmosphere, which will be used in metabolism. Each metabolic pathway produces a given end product that can either be the goal of the full chemical route, or a precursor for other processes. Any product achieved in each pathway used by other pathways is drawn in the same column where it has been produced. Thus, the length of each row represents the complexity of the pathway. Horizontal thick lines separate the global processes: (a) establishment of the central metabolic frame; (b) membrane synthesis; (c) total independence from the primordial soup by direct ATP production and glucose synthesis by means of CO_2 fixation; and (d) RNA synthesis and replication. *Abbreviations*: Fmal, formaldehyde; AdMet, Adenosyl-methionine.

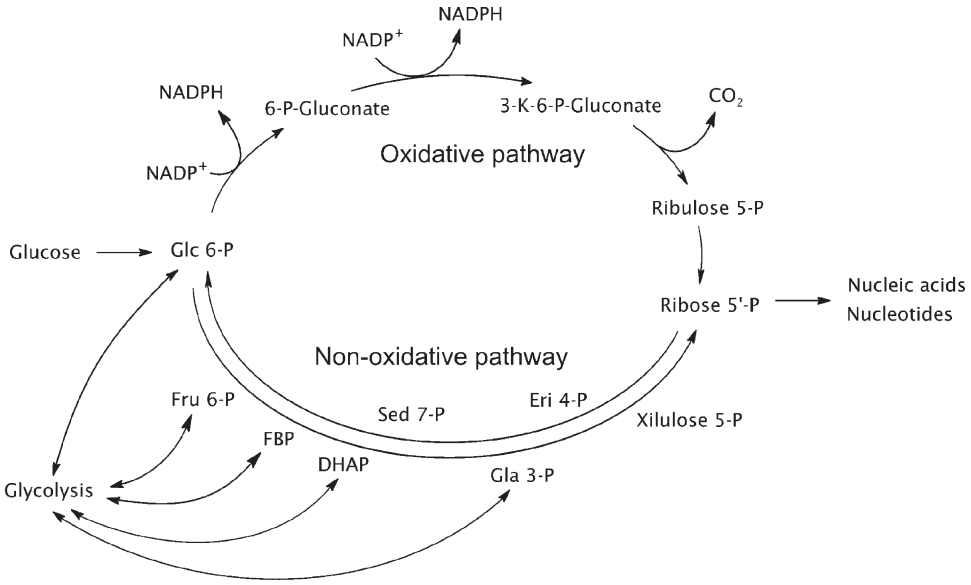

Fig. 4. The two pathways to convert glucose into ribose, and their coupling to yield the pentose-phosphate cycle, as a source of ribose and reductive power (NADPH), noting their relationship with the glycolytic pathway. It is important to see that the non-oxidative pathway is reversible, which gives a broad metabolic versatility to these sets of reactions. *Abbreviations*: DHA, dihydroxyacetone; Eri, eritrose; Fmal, formaldehyde; Gla, glyceraldehyde; Glca, glycolaldehyde; FBP, fructose 1,6-bis-phosphate; Fru 6-P, fructose 6-phosphate; DHAP, dihydroxyacetone-phosphate; Eri 4-P, eritrose 4-phosphate; Glc 6-P, glucose 6-phosphate.

coupled with the respiratory chain, was, thus a clear case of opportunism that could only be achieved when the aerobic conditions existed, which was not difficult, as all reactions of this branch are reversible (see Meléndez-Hevia et al., 1996). It is interesting to see that the horseshoe design of this pathway still remains in the present metabolism, as a good example of paleometabolism in some anaerobic bacteria (see White, 1995), and some anaerobic invertebrates (see Hochachka and Mustafa, 1972).

4.2.5. *Reductive power*

A hydrogen atmosphere can produce a reductive environment under alkaline conditions, as pointed out above. That reductive environment can allow certain important biosynthetic reactions, such as fatty acid synthesis, which is a critical goal for membrane synthesis. However, the reductive conditions that operated in the origin of life could not be very strong, because glycolysis—a primitive pathway—needs oxidative conditions to work, as the reaction that generates ATP is based on the oxidation of glyceraldehyde (for a discussion on its detailed mechanism see Meléndez-Hevia et al., 1997). Thus, a metabolic generation of reductive power independent of prebiotic conditions and separated from glycolysis reagents is a clear initial goal. Since the conditions must be oxidative for glycolysis, and reductive for biosynthetic reactions, the only possible solution is to have different redox coenzymes with different reduction potential to take charge of these different processes. Pyridine redox coenzymes as occurs in modern cells have a high standard reduction potential ($E_0' = -0.32$ V), which makes it a good reductive reagent but a bad oxidized reagent. However, this is modified in present metabolism by the concentration ratio between the oxidized and reduced species: the cytoplasmic ratios are around 10^3 for NAD^+/NADH (Williamson et al., 1967; Schwartz et al., 1974), which gives a lower real reduction potential ($E' = -0.23$ V) suitable for glyceraldehyde oxidation, and around 10^{-2} for $NADP^+$/NADPH (Kaufman and Nelson, 1981), which gives a higher real reduction potential ($E' = -0.38$ V) more efficient for reductive biosynthetic reactions. It is logical to think that in the premetabolic stage, these redox coenzymes were also different, and such feature has persisted at present.

4.2.6. *The pentose-phosphate cycle—ribose and fatty acid synthesis*

The two pentose-phosphate pathways are two alternative routes for producing ribose 5′-P, and their combination generates the pentose-phosphate cycle (see Fig. 4). Working with a cyclic design, the pathway is just a source of NADPH, as there is no draining of carbon from ribose. This cycle has a quality which is unique in all metabolic cycles: it does not need a specific anaplerotic pathway to supply the feeder, as it is the only case in metabolism in which both the substrate and the feeder are the same compound (glucose 6-P, as Fig. 4 shows). It could therefore appear early on as a source of NADPH for fatty acid synthesis, both processes taking the starting material from glycolysis. That the close coupling between these two processes has persisted into the present is a manifest example of metabolic continuity. Clear experimental data on this coupling can be seen in a previous work of our group (Cabezas et al., 1999).

4.2.7. Membrane synthesis

The basic membrane structure is a lipid bilayer. The primordial basic materials of lipids are fatty acids, which can be synthesized from acetyl-CoA and NADPH, whose origin has been explained in the previous section. As membrane lipids must have a high polar group, phosphatidyl-choline is an appropriate material as it is electrically neutral, thus, favouring the binding of the molecules to form the bilayer. Phosphatidyl-serine is also possible; it occurs, in fact, in some biological membranes, and it is obviously easier, as its biosynthetic pathway is shorter. Phosphatidyl-choline synthesis is also possible with the available material (see Fig. 3). Thus, membrane synthesis is a plausible process; moreover, this process is thermodynamically favoured, as the hydrophobic binding of the lipids is cooperative, and the combination of several different lipid species promotes the membrane asymmetry (Brumen et al., 1993; Heinrich et al., 1997c).

Lipid bilayers can spontaneously generate liposomes and then coacervates with a similar size to prokaryotic cells (10–100 μm diameter). Luisi et al. (2004) have experimentally demonstrated the size-reproduction of lipid vesicles, as when fresh surfactant was added to an aqueous solution containing preformed vesicles of a very narrow size, the newly formed vesicles have dimensions very close to those of the preformed ones. Thus, the next events of the chemical evolution could occur inside the coacervates, making the route to cellular metabolism easier as it will allow for the individuality of genetic information. Therefore, the hypothesis of Oparin (1938) on the role of coacervates in the origin of life is, indeed, plausible.

4.2.8. ATP synthesis and energy sources

The fact that ATP is the central molecule for energy traffic means that its first synthetic pathway was developed at the same time at the central core of metabolism, according to hypothesis of continuity. On the other hand, an interesting point of the metabolic map mentioned above, which must be especially considered for the purpose of this study, is that while the central pathways of carbon flux metabolism are universal, the ATP sources are not, as ATP can be produced by many different means: anaerobic glycolysis, other mechanisms of phosphorylation at the substrate level, oxidative phosphorylation, and different phosphorylation mechanisms dependent on photosystems. This obviously says that this diversity for obtaining metabolic energy was developed independently, but always converging in ATP.

There are many possible candidates for explaining the first ATP source from compounds that might be available in the primordial soup or from the mineral world. This could involve sulphur compounds such as pyrites (see, e.g., Maden, 1995), or the formation of thio-esthers from amino acids or other organic products, which can produce phosphate activated compounds, such as acetyl-phosphate, as they are indeed reactions that exists in the present metabolism (De Duve, 1991). Clarke and Elsden (1980) have suggested that some amino acids, such as glycine and proline, could have been involved in the earliest catabolic reactions, as it has been shown in *Clostridium sticklandii* by Stadtman et al. (1958). Clarke and Elsden (1980) suggest that some amino acid catabolic pathways of present-day anaerobic bacteria there may be traces of some of these primitive reactions, which in our scenario would be more cases of paleometabolism.

It is important to see that there is also a broad diversity not only in the mechanisms, but also in the primary energy source (photoautotrophy and chemoautotrophy). Our approach cannot, in principle, determine which of these mechanisms could be the first one to appear, nor can it state a sequence in time of the appearance of the diversity, as they are independent mechanisms. However, the hypothesis of continuity leads us to conclude that all these ATP pathways had to be developed after defining ATP as the central energy currency.

In many cases of present-day metabolism, the same photosynthetic processes generate both ATP and reductive power, both being used for the same purpose of carbon/nitrogen fixation. However, this cannot explain their occurrence in the same process before natural selection. The chemical explanation of this fact is that an oxidation to transfer electrons to $NADP^+$, or to make ATP, requires a very high reduction potential. Then, this system could produce ATP and/or NADPH. The fact that it is possible to make ATP from light energy without producing NADPH, as it occurs, e.g., in photophosphorylation coupled to cyclic electron transport, suggests that the first goal of these systems could be the generation of ATP, and that production of NADPH was a further opportunistic use of the electron transport involved in it.

In the same way that the generation of the energy currency (ATP) promotes biosynthetic metabolism, the generation of reductive power (NADPH) will promote the reductive metabolism leading to a number of biosynthetic pathways, and particularly, both to fatty acid synthesis from acetyl-CoA coming from glucose, as well as to glucose synthesis from CO_2 (or yielding acetyl-CoA directly from CO_2, by the Arnon cycle or by the Wood pathway). Thus, it is important to consider that one key principle in chemical evolution is that two different pathways, one of them producing ATP and/or NADPH, and the other one spending it, have not necessarily evolved at the same time; the first one could have occurred previously as it promotes the appearance of the second. This fact is important as it establishes a loose dependence between them, allowing a broad use of ATP and NADPH for other processes besides glucose synthesis.

4.2.9. Carbon photosynthetic pathways

The formose reaction (Fig. 2) and the structure of the metabolic map (Fig. 1) can explain that metabolism could start from glucose, but it was obviously necessary to develop a pathway capable of producing it from mineral material (CO_2 and H_2O), as a necessary step to divorcing metabolism from prebiotic chemistry. This process needs ATP and reductive power, and these two goals would

promote a further development of metabolism by producing glucose directly independently of the reductive environment, which could not be very strong anyway.

The same principle considered above on the diversity for ATP synthesis, applies here, as a number of different pathways exist in present-day metabolism to produce reductive power coupled to glucose synthesis. The close relationship between ATP producing pathways and synthesis of carbon and nitrogen compounds (involving the generation of reductive power) is obvious, showing that most of ATP pathways were coupled with biosynthetic processes. It is important to see that the development of these pathways is a clearly different from the development of the central carbon metabolism, because while this is universal, a broad diversity of ATP and autotrophic pathways exists. Therefore, our use of a retrospective analysis is not applicable to these particular pathways as a whole. It is possible, however, to apply our method to the development of each pathway individually, even though we cannot determine the sequence of their individual appearance.

A special feature of the Calvin cycle is that it is *autocatalytic,* as it can produce a net yielding of its feeder ribulose 5-P by: 5 ribulose + $5CO_2 \rightarrow 6$ ribulose. However, unlike the pentose cycle, it needs this product to start, so the pentose-phosphate pathways, as sources of it had to be built previously, as shown in Fig. 3.

On the other hand, the fact that the Calvin cycle uses NADPH instead of NADH, strongly suggests that a redox coupling between it and the pentose-phosphate cycle also existed in its origin, pentose-phosphate cycle not only being the source of ribulose 5-P, but also the source of NADPH. Considering this coupling from the point of view of carbon traffic, it is a futile cycle, as it does not produce a net CO_2 fixation, and it is also not a net source or expenditure of reductive power. However, it does account for a net conversion of sugars C_6 into C_5 (ribulose or ribose) without CO_2 loss by

(a) *Oxidative pentose pathway*:

$$5C_6\ (\text{glucose}) + 10NADP^+ \rightarrow 5C_5\ (\text{ribulose}) + 10NADPH + 5CO_2$$

(b) *Calvin cycle*:

$$5C_5\ (\text{ribulose}) + 5CO_2 + 10NADPH \rightarrow 6C_5\ (\text{ribulose}) + 10NADP^+\ (\text{autocatalytic})$$

Result : $5C_6$ (glucose) $\rightarrow 6C_5$ (ribulose).

The global stoichiometry of this process ($5C_6 \rightarrow 6C_5$) is the same as that of the non-oxidative pentose pathway, but the coupling of these two pathways gives a process with an important advantage because it is autocatalytic, generating six ribuloses from five. Thus, it is obviously favoured, which suggests that the Calvin cycle was coupled with the pentose pathway in its origin, and can explain why it uses NADPH instead of NADH.

The other two pathways for fixing CO_2: the Arnon (reductive reverse Krebs) cycle, and the Wood (acetate) pathway (see White, 1995) are independent routes that do not produce glucose directly, but acetyl-CoA, and so they need a very complex additional pathway to yield glucose. We discuss below the possible evolutionary significance of these pathways.

4.2.10. The RNA world

Information must be contained in macromolecules capable of autoreplication. Catalysts must also be macromolecules in order to have the necessary high specificity for being able to discriminate among a series of substrates that have a close chemical structure. However, it is not strictly necessary for catalysts to be different from the material that safeguard the information, i.e., it is not strictly necessary to separate molecular phenotype from genotype. Thus, since RNA can perform both functions, and since our purpose is the search for the minimal structures for natural selection to work, we can assume that the same RNA macromolecules can work covering both purposes.

It is important to note that this is really possible, as RNA plays as informative material in RNA viruses, and it is also well documented that RNA can do as enzymes (ribozymes) catalysing several reactions (Grasby, 1998), particularly, ribosomal RNA plays a pre-eminent role as catalyst of reactions of protein biosynthesis (Steitz and Moore, 2003). We can assume, thus, that life could start with the RNA world, as it agrees with the minimal conditions for life emergence (Grasby, 1998; Steitz and Moore, 2003; Orgel, 2004).

The pass of the RNA world to DNA-protein world means the separation of genotype and phenotype, involving profound changes of material. Although the study of the possible sequence of such changes is far from the aim of this work, it must be understood that they were really possible as natural selection already operated. Thus, RNA

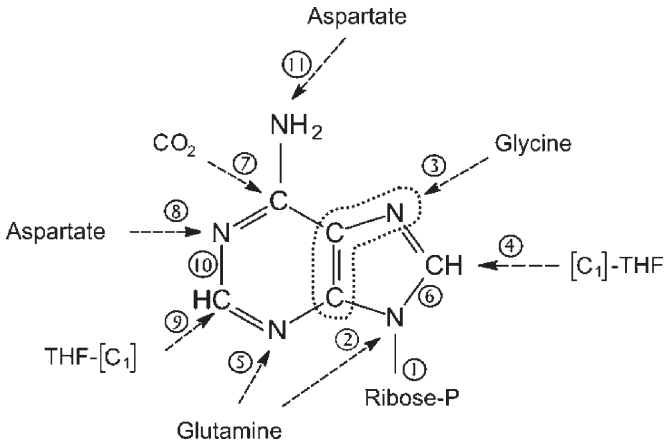

Fig. 5. Structure of one purine basis (Adenine), showing the metabolic origin of each atom, and the order in which the molecule is built in metabolism. Numbers indicate the order of the reactions in the process of ring construction (incorporation of the atoms and formation of the bonds). The sequence of the steps is chaotic with no logic, which suggests that its origin was a random mixture of reactions, typical of the prebiotic chemistry, until achieving a very stable molecule.

molecules could codify both protein and DNA synthesis, preserving the catalytic information.

4.2.11. Nitrogen metabolism

Nitrogen fixation is not difficult in this early metabolism as ammonia is readily available. The goal is achieved by means of a simple reaction with α-ketoglutarate and NADPH that yields glutamate. Then glutamate can direct all nitrogen traffic towards amino acids and phospholipids, these processes having remained fixed until present. The early metabolism could be therefore designed based on ammonia as it was abundant in the primitive atmosphere, and so establishing the dependence on this product. Then, further development of metabolism requires to have an own NH_3 source from mineral nitrogen in order to make it independent of that primitive atmosphere. This could be achieved by several pathways, all of them existing in the present-day metabolism, that produce NH_3 from N_2, NO^{2-} or NO^{3-}. NH_3 synthesis is made in present-day metabolism by nitrogenase with a very complex catalytic mechanism, but it is also possible just with iron as catalyst as it occurs in Haber's synthesis (see Friend, 1993). In fact, iron plays a pre-eminent role in the catalysis mechanism of nitrogenase (Leigh, 1998).

The pathway of purine nucleotides is perhaps the oddest pathway in metabolism. The building of the purine ring is carried out using many different materials without an obvious, logical chemical synthesis design (see Fig. 5), like if these chemical reactions had been randomly assembled, as it could occur in prebiotic chemistry. Moreover, most reagents used in the synthesis of the purine ring are elementary compounds present in the primordial soup: NH_3, CO_2 and formic acid—as glutamine and aspartate only participate as amino donors. Glycine, whose molecule participates in full, is the only exception, but it is precisely the most abundant product formed under prebiotic conditions (see Miller, 1995). This suggests that this process could be a good example of a biosynthetic pathway brought directly to metabolism from prebiotic chemistry. Although the process of the purine ring synthesis is complex, the end aromatic product is very stable, which can explain its spontaneous formation in prebiotic chemistry, experimentally demonstrated by Oró and Kimball (1961). Pyrimidine ring synthesis is not difficult if aspartate and carbamoyl-P are readily available, as these are the basic material for this type of synthesis and, as the ring is also an aromatic compound, it is also very stable. Activation of nucleotides is not difficult once ATP is present. RNA production is then much easier, because nucleotide polymerization is very exergonic; furthermore, it is cooperative, and its replication is autocatalytic.

Prebiotic synthesis of several amino acids, especially glycine, might suggest that this or other similar compounds could have any role as a starting point of metabolism. However, this presumption is not very plausible. Glycine and some other amino acids can have been products of prebiotic chemistry, and, in fact, their synthesis pathways, or some parts of them, might have been conserved to the present metabolism, as it most likely occurred with purine synthesis. However, their relevance as starting points of the present metabolism is unlikely, as it is clear that the metabolic map has not been built on them, they being only end points of it. Their relevance can be only, thus, as isolated parts of the metabolic map that have persisted, but not starting points, differing of glucose, whose relevant role is so clear.

4.2.12. External catalytic mechanisms

Apart from the possible role that some prebiotic products could play in the origin of metabolism, several amino acids, such as serine, alanine, cysteine, asparagine, or proline, could be produced as by-products, as well as some others, such as glycine, aspartic acid, α-alanine, β-alanine, and α-amino butiric acid, which have been found as elementary products present in prebiotic chemistry (Miller, 1953, 1955). It is therefore possible that the spontaneous random polymerization of these amino acids (which is chemically possible, even without ATP expenditure (see, e.g., Fox, 1960; Chang et al., 1969; Leman et al., 2004)) yields small peptides capable of catalysing many reactions (Weber and Pizzarello, 2006). We can assume that most of such catalysis was not specific (although some of it has been shown to have some specificity; see Weber and Pizzarello, 2006). On the other hand, that peptide synthesis at this stage had no continuity as it was not codified into informative material. However, its effect could be important, increasing the rate of all chemical reactions, without participating in chemical selection, but accelerating it (and so, the selected processes) before the appearance of RNA. This effect of unspecific peptide catalysis could be added to the clay catalysis described by Cairns-Smith (1982) in enhancing the rate of the chemical reactions considered in this work, so favouring the chemical selection.

5. Discussion

The materials necessary for natural selection had to be achieved beforehand, by 'chemical selection'. As this process operates through selecting the reagents and reactions independent of their Darwinian selective value, it can only be driven by increasing the rate of the reactions as a consequence of their own chemical features. Thus, in this process, catalytic, autocatalytic and cooperative effects, as well as thermodynamic driving by available substrates or forces, such as energy currency and reductive power that can favour certain reactions, could have played a critical role, enhancing their probability.

The set of reactions shown in Fig. 1 is the simplest one able to justify the appearance of the basic materials needed for the emergence of life, according with the hypothesis of metabolic continuity until present-day metabolism. These reactions are now catalyzed by enzymes, but in the RNA world, at the beginning of life, they would be catalyzed by RNA. As this scheme has been built only considering

reactions that exist nowadays in cellular metabolism, it fulfils the hypothesis of continuity, accounting for a further emergence of enzymes. Thus, enzymes could appear later, substituting RNA progressively by natural selection, promoting the separation between genotype and phenotype, and so creating the 'protein word'. Cairns-Smith (1982) has pointed out a number of criticisms on the implausibility of prevital nucleic acid. We agree basically with those objections, as our model does not assume the existence of RNA before metabolism, but after a chemical evolution based on specific thermodynamic and kinetic forces, and a further specialization of it to catalyze the selected chemical reactions.

The sequences of events presented in Fig. 3 show the dependence of each process on the precedent ones in each row, according to the approach of retrospective analysis. It means, for instance, that fatty acid synthesis occurred after glycolysis (as the source of acetyl-CoA), and also after the oxidative pentose pathway (as the source of NADPH). The length of each row shows the complexity for building the pathway. On the other hand, the fewer the new substrates needed, the more opportunistic the pathway. These two features (complexity and opportunism) have been important in the emergence of each new pathway, but this rule does not necessarily determine a given sequence of facts, because increasing its probability of occurring does not means that it must occur first obligatorily. In fact, random events have played an important role in evolution provoking stochastic results. For example, although our analysis shows that the Calvin cycle is a pathway more opportunistic and less complex for fixing CO_2 than the other two procedures (the Arnon cycle and the Wood pathway), as Fig. 3 shows, data from molecular phylogenetic trees show that it was the last one to appear (Peretó et al., 1999). Our results also show that this is possible because these three pathways are independent, as they use different substrates as starters, besides CO_2, ATP and NAD(P)H.

Which the first metabolic pathway was, including the first ATP synthesizing pathway, as well as which the sequence of pathways emergence was, is a subject continuously submitted to a high controversy. It is important, first of all, at this point to remark that our approach to determine that, leading us to its origin, is based on two main principles: opportunism and continuity. It is important to consider that continuity of metabolism, which is a basic evolutionary rule, leads to the fact of universality of central carbon pathways, as well as to distinguish the particular pathways that could be later incorporated to the central one. Thus, it could be possible to derive the sequence of events in the evolution of each branch, as well as its connection with the central pathways, but it is not possible obviously with this approach to know a hierarchy in the emergence of these branches pathways, as they can have appeared independently. Morowitz et al. (2000) have proposed that the Arnon cycle could be the origin of metabolism. The problem of this proposal is its lack of continuity, as it only exists in some bacteria, and so it cannot account for the building of the metabolic map, as then, the starting substrate in the map would be acetyl-CoA with a system to convert it into pyruvate (a ferredoxin dependent system that only exists in bacteria). Thus, the Arnon cycle is rather a good example of the diversity of the CO_2 fixation pathways, which is independent of the establishment of the central carbon pathways where glycolysis is the main skeleton. The case of the pathways of synthesis of some amino acid, such as glycine, is different: these pathways are universal; thus, although their role as the origin of the general metabolic map is unlikely as we have discussed above, their presence in the universal map suggest that they could be designed in the early states of chemical evolution, independently from glycolysis as particular parts of the metabolic map and were later connected to it at the appropriate points. Thus, following Reinhart Heinrich's suggestion mentioned in the introduction, about comparing the structure of metabolism with the structure of a city, in order to know the history of its origin, we can conclude that metabolism was built by radial growth from a central nucleus (glycolysis and the horseshoe Krebs cycle), but also by joining other parts to it, a last result that would be Heinrich happy but that unfortunately he could not know. The analysis of the specific amino acid metabolism pathways could give us information about the pathways that can have been originated as branches starting from the central core, and the ones that can have been built independently and later be joined to it. This is a task that we shall make in a further work.

The sequence of the pathways appearance presented here can justify the appearance of natural selection, but does not imply that the appearance of the enzymes must be follow this same sequence. Natural selection can operate on any point, and it always can enhance this primitive scheme. The purpose of this paper was to find a logical sequence of events that could account for natural selection emergence having continuity with the present metabolism.

Results of the analysis presented in Fig. 3 show that the three main objectives for the emergence of life, namely membrane synthesis, metabolism (including photosystems as definitive source of ATP and NADPH, and glucose synthesis from CO_2), and RNA synthesis and replication, can be achieved independently by chemical selection, as all of them are independently catalytic, autocatalytic and/or cooperative, and furthermore, all of them are thermodynamically favoured by the prominent previous production of their substrates (mainly ATP and NADPH). In this process, membrane synthesis, as perhaps the easiest end, could be one of the first achieved goals. This could highly favour all further chemical development including of course RNA synthesis and its replication, which would have taken place inside the coacervates. Thus, once the difficulties (through pure chemistry) have been surmounted, the rules of life at last prevail, and the next steps for completing the full metabolic map—including the appearance of the protein enzymes, organic coenzymes, and the genetic code—must be much easier, because at this point, natural selection is possible, and so life can begin.

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