Nontemplate-driven polymers: clues to a minimal form of organization closure at the early stages of living systems

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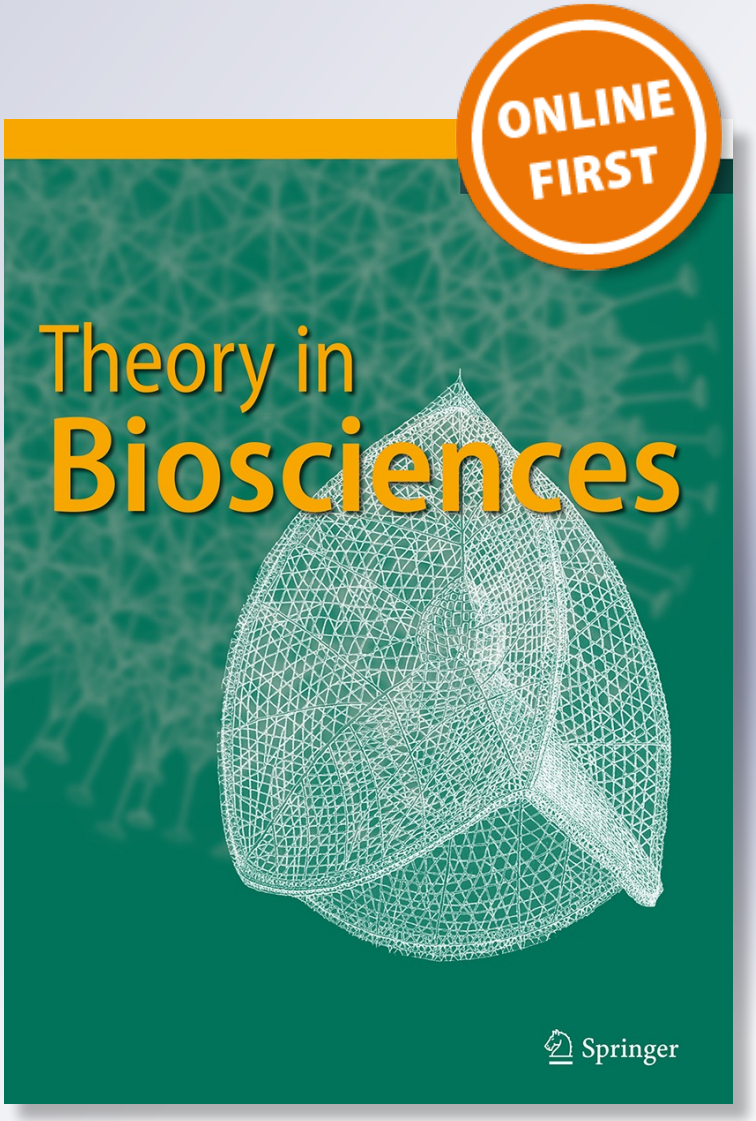

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ORIGINAL PAPER

Nontemplate-driven polymers: clues to a minimal form of organization closure at the early stages of living systems

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Abstract The emergence of the first polymers played an essential role in the transition from the physicochemical to the biological domain, a perception that embodied many different world paradigms relying on only one primal polymer. However, biological complexity would have appeared with an increasing set of associated chemistries and molecular interactions of many different macromolecules. In agreement with this notion, here, the purpose is to focus specific attention on current knowledge of modern biochemistry of a set of widespread polymers likely present in the Last Universal Common Ancestor synthesized by nontemplate-driven reactions with references to their abiotic synthesis. The proposed overview describes the manner in which these polymers could have organized around two polymerization reaction cycles and integrated into a minimal organizational closure at the early stages of living systems, independently of template replication processes. This hypothesis could provide an alternative conceptual framework to evaluate a plausible scenario addressing the transition from nonliving to protocellular systems.

Keywords Origin of life · Organizational closure · Emergence · Macromolecular worlds · Protometabolism · Polynucleotide phosphorylase · Q-Beta replicase · Degradosome

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Introduction

A high degree of controversy characterizes the study of the origin of life. However, scientific hypotheses posit on the philosophical assumption that there is no gap between inorganic matter and living systems, called "the continuity thesis" (Fry 1995). Concerning the transition from merely physicochemical to properly biological systems, Bernal (1959) stated, "The first crucial step, which enabled life to get beyond this stage and to emancipate itself from mineral support, was the production of polymers".

The free energy gradients available to drive monomer condensation reactions that deserve major attention in evolutionary context are those that remained constant on geological timescale. Among them, continuous energy fluxes which were needed for the first synthesis may have been provided by solar radiation (UV light and heat) and geochemical sources (heat and mineral reducing power). Another occasional energy inputs from impact collisions, atmospheric electric discharges, shock waves, volcanic explosions might have also contributed to the emergence of reacting molecules which in turn would produce more complex compounds. Within the chemical sources of energy thioesters, pyrophosphate carriers and abiotic synthesized sugars could also serve to activate primitive polymerization pathways (Deamer and Weber 2010).

Nowadays, life depends on both metabolic and self-replication activities based on polymers that are stored and consumed or expressed, reproduced and transmitted to the progeny. The self-maintenance and transmission of the information (genetic memory) is ensured by nucleic acids and proteins synthesized by template-dependent polymerization reactions (genetic system). The rest of biopolymers and all other biological compounds are synthesized by nontemplate-dependent processes. They do not transmit

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genetic information. However, memory can be instantiated in chemical networks, catalytic loops and reflexive autocatalysis repeated indefinitely. These cycles could also generate distinguishable populations of uncoded replicators with different chemical processes, efficiencies and physicochemical properties (Morowitz 1999).

One of the most basic questions in the origin of life is to know which polymers could have first evolved. Many proposals state the relevance of polymers prior to the emergence of living systems on Earth with emphasis on only one kind of macromolecule. A primal role for a glycoworld in the beginnings considers the natural propensity of carbohydrates to polymerize (Weber 2005), to act as compatibilizers and concentrating agents of other polymers under physicochemical constraints (Tolstoguzov 2004), and scaffold for the assembly of other macromolecules (Stern and Jedrzejas 2008). The RNA-world hypothesis proposes that life began with replicating entities (Gilbert 1986). In the Fox's "microspheres" proposal, amino acids combined to form proteinoids, and in turn, proteinoids formed small cell-like spheres (Fox 1980). The lipid world scenario posits on the potential catalytic activities of lipids and other amphiphiles with their capacity to undergo spontaneous self-organization into supramolecular structures such as micelles and bilayers allowing the emergence of compartmentalization and cellularity (Segré et al. 2001).

Nevertheless, the explanation of origins requires a holistic integration into more complex scenarios of many different components.

For proponents of theories on the origin of life based on a metabolic-first scenario, life could have started from abiotic chemical reactions and nontemplate-dependent processes separated from the environment. For example, Oparin and Fox hypothesized that earliest life-like properties could have been materialized without the need of membrane boundaries through the formation of increasingly complex organic structures called "coacervates" (Oparin 1957) or "proteinoid microspheres" (Fox 1980), respectively. Other metabolism-first theories suggest a self-organized set of small molecules and uncoded polymers (oligopeptides) originated from autocatalytic processes that become replicative later (Kauffman 1993).

Replication-first theories embedded in the RNA-world hypothesis propose that life began with replicating entities (replicators) capable of both self-reproduction and simple metabolism that constituted a genetic code (Crick 1968; Gilbert 1986). Joyce (2002) suggested an RNA-later hypothesis in which simpler polymers emerged by prebiotic chemistry and evolved to RNA.

However, replication-first proposals seem constrained by assuming the appearance of replicator(s) as self-copying and template polymer(s) (Shapiro 2000). A simpler scenario is to consider that the first replicator(s) would have emerged from nontemplate-dependent processes, to later on become replicative; a possibility illustrated by polynucleotide phosphorylase (PNPase) capable of synthesis and degradation of RNA by template-free reactions (Freire 2011).

Before the discovery of nucleic acid polymerases, the first hypothesis presumed a functional similarity with glycogen phosphorylase (Kornberg 2001), an enzyme of metabolism of carbohydrates involved in degradation of glucogen that is capable of glucose polymerization in vitro. Identification and characterization of polynucleotide phosphorylase, a nontemplate-dependent ribonucleotide polymerase (Grunberg-Manago and Ochoa 1955) and other nucleic acid polymerases reported in the early 1960s provided experimental evidences supporting the initial premises. According to in vitro synthesis of polyribonucleotides, Ochoa was inclined to call it RNA synthase, while Grunberg-Manago suggested polynucleotide phosphorylase to reflect its analogies with glycogen phosphorylase in the reversible synthesis of polysaccharides (Grunberg-Manago 1989).

Moreover, Mirsky (1959) discussed the evolution of nucleic acids in a note presented at the First International Symposium on the Origin of Life in 1957 at Moscow convened by Oparin and the Soviet Academy of Sciences. He stated, "The fact that small, relatively simple polynucleotides can function in ATP synthesis is of interest from the evolutionary point of view. It may be that it was the original role of the polynucleotide structure and that the evolution of the cell brought with it a parallel molecular evolution in which these nonspecific polysaccharide molecules were gradually modified to take on a new complexity and assume a new function: a role in the transmission of hereditary specificity". However, apart from the proposed transition of polysaccharides to polynucleotides, the functional similarities found between glycogen phosphorylase and polynucleotide phosphorylase was not sufficient to argue in favor of a plausible framework linking the emergence of polysaccharide and polyribonucleotide polymers (Freire 2011).

Quite to the contrast, another enzyme, the viral Q-Beta replicase attracted much more attention. From its discovery (Haruna and Spiegelman 1965), it showed to be capable of specific synthesis for its corresponding genomic RNA. Even more, Sumper and Luce (1975) discovered that a mixture containing no RNA at all but Q-Beta replicase could generate self-replicating RNA molecules in vitro. Self-replicating activities allowed competitive RNA replication experiments, the first in vitro molecular evolution assays and led to develop scientific research programs to explore the origin of biological information based on two vital concepts: quasi-species and hypercycle models (see below) (Spiegelman 1971; Eigen 1971; Eigen and Schuster

1977; Eigen et al. 1988). However, Oparin and other authors argued that such systems would result only in the accumulation of replicating molecules but not lead to a biosphere (reviewed by Lazcano 1986). In keeping with this regard, Oparin and co-workers who knew the work of Grunberg-Manago and Ochoa, used both polymer phosphorylases: potato phosphorylase to study the enzymatic polymerization of glucose-1P (Oparin et al. 1962) and polynucleotide phosphorylase in the polymerization of ADP (Oparin et al. 1963) in coacervates as models of prebiotic cells. In further steps toward model cells, both Chakrabarti et al. (1994) and Walde et al. (1994) reported that encapsulated polynucleotide phosphorylase in lipid vesicles and micelles could synthesize an RNA homopolymer from ADP.

Conceptual outline

Previously, an overview showed that although glycogen phosphorylase and polynucleotide phosphorylase have no apparent homology, they share striking properties and features that may reflect deep commonalities in the emergence of polysaccharide and polyribonucleotide polymers connected with polyphosphates through intertwined non-replicative reactions (Freire 2011). Here, the purpose is to extend that hypothesis to an evolutionary history of pre-cellular organization including other nontemplate-driven polymers that might have occurred in the early scenarios of life. Geochemical pathways would have favored the formation of reactive monomers and polymers such as polyhydroxyalkanoates, polyphosphates, polysaccharides, polyribonucleotides, fatty acids, polyisoprenoids and peptides underlying a minimal organizational closure that further allowed the emergence of more complex systems. Concerning to the organizational closure applied to biological systems, Mossio (2013) proposes the following definition: "Closure refers to a holistic feature such that their constitutive processes, operations and transformations (1) depend on each other for their production and maintenance and (2) collectively contribute to determine the conditions at which the whole organization can exist". Organization closure is a self-referential system that does not require input from the environment for maintaining itself.

Brief summary of metabolism and carbon assimilation

Glycolysis and gluconeogenesis, pentose phosphate pathway and tricarboxylic acid (TCA) cycle are central metabolic pathways. Their products glucose, pyruvate, ribose-phosphate and erythrose-phosphate are common precursors for amino acids, lipids and nucleotides.

Pentose phosphate pathway generates NADPH and pentoses from glucose-6P. Glycolysis is the most common sequence of reactions for the conversion of glucose-6P into pyruvate. It yields ATP, reduced equivalents, and precursor metabolites for a multitude of essential cellular processes. Gluconeogenesis is the reverse pathway that results in the generation of glucose from non-carbohydrate carbon substrates such as lactate, glycerol, and glucogenic amino acids. Glycolysis, glycogenesis and the pentose phosphate pathway overlap and share most reaction pathways with the Calvin cycle. Glycolysis and pentose reaction pathway could occur non-enzymatically under conditions that replicate those of the Archean ocean catalyzed by metal ions (Keller et al. 2014).

Carbon assimilation from inorganic (autotrophic pathways) or organic compounds (heterotrophic pathways) played an essential role in the origins of life, but which mechanism could have first evolved is a matter of debate.

The reactions and compounds of the tricarboxylic acid cycle, the core of carbon metabolism are universal in modern organisms and five compounds (acetate, pyruvate, oxaloacetate, succinate, and α-ketoglutarate) constitute the building blocks from which anabolic pathways originate (Srinivasan and Morowitz 2009).

There are at least six known autotrophic carbon-fixation mechanisms (Berg et al. 2010; Fuchs 2011; Braakman and Smith 2013). Yet, even though knowledge about their phylogenetic distribution is substantial, it is unclear if all these pathways operated in the LUCA (Nitschke and Russell 2013).

The Calvin–Bassham–Benson cycle is present in photosynthetic organisms and the predominant mechanism in the biosphere. RuBisCO converts ribulose-1,5-biphosphate plus CO_2 and water to two molecules of 3-phosphoglycerate.

The other five carbon-fixation biochemical mechanisms are present in chemoautotrophic organisms and serve as modern models of emergence and evolution for primordial intermediary metabolism. They include: the reductive acetyl-CoA pathway or the linear Wood–Ljungdahl reaction; the reductive tricarboxylic acid cycle (rTCA); the dicarboxylate/4-hydroxybutyrate cycle; the 3-hydroxypropionate bicycle and the 3-hydroxypropionate/4-hydroxybutyrate cycle.

All fixation pathways, with the exception of the Calvin–Bassham–Benson cycle, result in the formation of acetyl-CoA, regarded as the turntable of metabolism interconnected with many metabolic links: alcohols, polyhydroxybutyrates, alkanes, fatty acids and waxes, among many others (Fuchs 2011; Fuchs and Berg 2014).

Overview of hypothesis about protometabolism

The chemical reaction cycles that might have allowed the formation of the first metabolic systems remain poorly constrained, and a large number of plausible scenarios have been envisioned.

Bernal (1959) proposed a pattern of stages of increasing inner complexity following one another in order of time, each one including structures and processes evolved at the lower levels. With regard to the supply of organic sources consisted mainly of water, carbon dioxide, ammonia, and hydrogen sulfide. In his view, biochemical approaches would search in the inorganic world for the origin of the organic world, and attempt to establish the correct order of the steps inferred from the existing metabolism.

Hartman (1975) explains the metabolic evolution from a simple environment by assuming that autotrophic pathways were involved in the fixation of CO_2 and N_2 present in a secondary atmosphere, and pointed out the centrality of anabolic functions played by the Krebs cycle followed by amino acids, lipids, nucleotides and carbohydrates. The first polymerization would have been the synthesis of fatty acids from acetyl-CoA.

In the context of an iron-sulfur world, the autocatalytic anabolist theory (Wächtershäuser 1990), assumes a reductive citric acid cycle that used thio acids, the reducing power obtained from the oxidative formation of pyrite (FeS_2) concatenated with homologous cycles for the synthesis and degradation of phosphorylated sugars, some amino acids, fatty acids, coenzymes and purines.

Morowitz (1999) who considers that modern metabolism recapitulates prebiotic chemistry and that anabolism is functionally prior to catabolism, proposed the expansion of biomass through sequential addition of concentric metabolic shells. The first shell consisted of glycolysis, the citric acid cycle and the pathways of fatty acid synthesis. Once established, this shell allowed the synthesis of long-chain hydrocarbon precursors to amphiphiles and provided the energy compounds such as polyphosphates and reductants. Hexoses and pentoses also synthesized in the first core shell. Morowitz postulates an autotrophic origin where a reductive citric acid cycle (or Arnon cycle) that took environmental CO_2 to make carbon compounds would have been the engine of synthesis of the major classes of biomolecules even preceding cellularity.

By retrospective analysis, Meléndez-Hevia et al. (2008) suggest a metabolism built from glucose synthesized by the autocatalytic and unselective formose chemical reaction that gave products with varied number of carbon atoms. Through glycolysis as the first starting point of a primitive metabolism, yield energy and building blocks (glycerol, pentoses, and oxalacetate among others) coupled with a horseshoe or incomplete Krebs cycle from which all other synthetic pathways were branched.

Calvin (1961) obtained by abiotic photoreduction of CO_2 diverse organic compounds and suggested a possible scenario for the origin of life. Mulkidjanian et al. (2012) suggest that the first protocells were heterotrophs. Their growth depended on the supply of organic compounds produced by abiotic photosynthesis. Photoreduction could have driven CO_2 fixation catalyzed by ZnS and MnS particles at the earliest stages of life (Mulkidjanian 2009).

According to de Duve (1995, 2003), life emerged through four successive worlds: the primeval prebiotic, the thioester, the RNA and finally the DNA worlds. The thioester world hypothesis is based on that thioesters could have served as a source of energy before the advent of ATP, and intermediates (like acetyl-CoA) in several key processes in the synthesis of fatty acids, glycerides and other esters, peptides, polyketides, sterols, isoprenoids, porphyrins, citrate and malate that established a prebiotic protometabolic network.

Biosynthesis and degradation of nontemplate-driven polymers

Polyhydroxyalkanoates

Among the polyhydroxyalkanoates, the poly-3-hydroxybutyrate (PHB) is the most abundant. PHB is a stereoregular, amphiphilic and linear homopolyester of (*R*)-3-hydroxybutyrate. PHB is present in prokaryotes and eukaryotes formed from acetyl-CoA. In bacteria, the processive synthesis occurs in three steps. First, the condensation of two molecules of acetyl-CoA forms acetoacetyl-CoA by a reversible reaction (condensation or thiolysis) catalyzed by β-ketothiolase enzyme with release of CoA. This intermediate reduced by NADPH forms (*R*)-3-hydroxybutyryl-CoA by the stereo-selective acetoacetyl-CoA reductase. Then, (*R*)-3-hydroxybutyryl-CoA polymerizes by PHB synthase with release of CoA. Models for the initiation mechanism involve self-priming with (*R*)-3-hydroxybutyryl-CoA with the growing polymer covalently attached to the synthase (Stubbe et al. 2005; Reusch 2013).

Bacterial PHB-containing granules known as carbonosomes are reserve of carbon and energy that accumulate under conditions of nutrient imbalance, when carbon sources are available but other nutrients for example nitrogen are limited. Under unrestricted growth, depolymerases degrade PHBs and the monomers are used as

building blocks and source of energy in the biosynthetic pathways (Stubbe et al. 2005).

Synthesis of polyhydroxyalkanoates requires acetate and reducing potential, both assumed available at early stages of life. An integrated overview of the regulation of cyclic polyhydroxybutyrate production shows its relationship with carbon-fixation pathways (tricarboxylic acid cycle) and acetyl-CoA (Stubbe et al. 2005).

Biosynthesis of polyhydroxyalkanoates

$$2\ \text{Acetyl-CoA} \rightarrow \text{Acetoacetyl-CoA} \rightarrow \text{polyhydroxyalkanoate}$$

Polyphosphates

Polyphosphate is a linear polymer of many hundreds of orthophosphate (Pi) residues linked by phosphoanhydride bonds and occurs in the three domains of life. Polyphosphate kinase (PPK) is an ADP phosphotransferase capable of catalyzing the processive polymerization of the terminal γ-phosphate of ATP into a polyphosphate chain and yields ADP. In the reverse reaction, the enzyme accepts all nucleoside diphosphates and uses a polyP chain as phosphate donor to produce NTP (Kornberg et al. 1956; Achbergerová and Nahálka 2011). In vitro synthesis of polyP by PPK at low ATP concentrations shows a lag that is suppressed by addition of poly$(P)_4$ oligomer that functions as a primer. There is an autophosphorylation of the enzyme under the conditions of polyP synthesis (Ahn and Kornberg 1990). However, it is unknown if a chain or protein functions as an initiator in vivo.

Exopolyphosphatase is another enzyme involved in polyphosphate degradation, releasing processively Pi from the ends of polyP (Kornberg 1995).

Structural analysis of bacterial PPK showed similarities with other polymerases such as RNA polymerases in that it synthesizes the polyP chain inside the tunnel where the active site is located (Zhu et al. 2005), and led to the idea that PPK is a polymerase without template. Two magnesium ions coordinate with the three phosphate groups of ATP at the ATP-binding pocket. Two metal ions mechanism is a common feature observed in many nucleic acid polymerases (Steitz 1998).

Bacteria, in response to protein-unfolding oxidative stress redirect ATP to polyP that stabilizes proteins in vivo and prevents their irreversible aggregation. In vitro, polyP has protein-like chaperone activities, binds unfolding proteins in an ATP-independent manner. Upon relief of stress polyP reconverts to ATP by PPK (Gray et al. 2014).

PolyP has a large capacity for cation exchange because of its high degree of rotational flexibility and negative charges; it shows preferential adjustment for binding divalent cations at physiological pH. However, it is unable to penetrate the lipid bilayer due to the high charge density. On the other hand, the amphiphilic nature of PHB with repeating units of hydrophobic methyl groups and hydrophilic esters form a structure surrounding the core helix of polyP with Ca^{2+} bridging both polymers. These polyP/PHB complexes form voltage-dependent ion channels positioned across membranes. The association of PHB with polyP would avoid Ca^{2+} precipitation, allowed its transport and facilitated the movement of nucleic acid polymers (Reusch 1999).

Kornberg (1995) proposed that polyP represents a fossil of prebiotic evolution as recycling source of energy, phosphate reserve, chelator of metal ions, a buffer against alkali, channel for DNA entry, and regulator for stress and survival among others. Moreover, polyP facilitates the nonenzymatic polymerization of carbohydrates to polysaccharides, nucleosides to nucleic acids and amino acids to polypeptides (Schramm et al. 1962).

PolyP is present in polyphosphate bodies, historically described as volutin granules, distributed in a variety of microorganisms including bacteria, archaea, algae, and protozoans (Kornberg 1995), and later identified as acidocalcisome, an energy-harvesting organelle rich in polyP, pyrophosphate, orthophosphate and calcium (Vercesi et al. 1994). Recently, phylogenetic and protein domain analysis of the membrane-bound vacuolar proton translocating pyrophosphatase and electron microscopy studies suggest that the acidocalcisome organelle may have originated prior to the divergence of the superkingdoms, highlighting the centrality of the cellular compartmentalization and bioenergetics properties of these ancient vesicles probably already present in the LUCA (Seufferheld et al. 2011).

Biosynthesis and degradation of polyphosphates

(a) Polyphosphate kinase

$$\text{PolyP}_n + \text{ATP} \rightleftarrows \text{polyP}_{n+1} + \text{ADP}$$

(b) Exo polyphosphatase

$$\text{PolyP}_n \rightarrow \text{polyP}_{n-1} + \text{Pi}$$

Polysaccharides

Glycogen phosphorylase is a glycosyltransferase that catalyzes the phosphorolysis of α-glucans and leads to the formation of glucosyl 1-P by glycogenolysis. In vitro, the enzyme is capable of synthesizing glycogen with a lag abolished by adding small amounts of glycogen (Cori and Cori 1939). In vivo, glycogenesis proceeds through glycogenin that acts as priming protein autocatalyzing the initiation step by self-glucosylation of about 7–11 units of

glucose attached to a tyrosine residue. Other enzyme, the glycogen synthase catalyzes the elongation of the linked chain using sugar residues combined with ribonucleoside diphosphate as glycosyl donor (Lomako et al. 2004).

Chemolithoautotrophic organisms serve as models for the study of primordial metabolic requirements because they synthesize all metabolites from inorganic precursors using only the core and anabolic pathways. These organisms capture carbon dioxide through diverse fixation pathways generating acetyl-CoA from which gluconeogenesis might begin (Berg et al. 2010).

Distribution and phylogenies of the enzymes of the glycolysis Embden–Meyerhof–Parnas pathway support a gluconeogenic origin of metabolism (Ronimus and Morgan 2003). Recently, biochemical assays with extracts obtained form thermophilic archaea and phylogenetic analysis allowed the identification of a highly conserved bifunctional fructose 1,6-biphosphate aldolase/phosphatase. This enzyme catalyzes the unidirectional conversion of two triosephosphate molecules directly to fructose 6-phosphate and inorganic phosphate. It might represent an ancestral gluconeogenic enzyme (Say and Fuchs 2010).

Bacterial polyP-glucokinase can attack polyP at its terminal residues. It catalyzes the phosphorylation of glucose to yield glucose-6P using ATP and/or polyP as phosphoryl donor in glycolysis. Glucokinases have no apparent homology but probably an ancestral relationship to the hexokinases found in Eukarya (Ronimus and Morgan 2003). PolyP-glucokinase reaction is present in a number of microorganisms and considered as a fossil reaction of primitive glycolysis (Kulaev et al. 2000). On the other hand, the other enzyme polyP kinase catalyzes UTP regeneration and enables the synthesis of oligosaccharides (gluconeogenesis) in vitro (Noguchi and Shiba 1998).

Biosynthesis and degradation of polysaccharides

(a) Glycogen phosphorylase

$$(\text{glucosyl})_n + \text{Pi} \rightleftarrows (\text{glucosyl})_{n-1} + (\text{glucosyl 1} - \text{P})$$

(b) Glycogen synthetase

$$(\text{glucosyl})_n + (\text{glucosyl} - \text{ribonucleoside} - \text{PP}) \rightarrow (\text{glucosyl})_{n+1} + (\text{ribonucleoside} - \text{PP})$$

Polyribonucleotides

Most of the template-independent RNA polymerases belong to the polβ superfamily of nucleotidyltransferases that catalyze the polymerization of nucleoside triphosphates. These enzymes, known as RNA-specific ribonucleotidyl transferases, include CCA-adding enzymes, polyA polymerases, uridyl transferases and oligoA-synthetases, and have the same fold in the catalytic domain (Martin and Keller 2007).

Polynucleotide phosphorylase (PNPase) is a nucleotidyltransferase with distinct scaffolds, capable of catalyzing the processive nontemplate-dependent polymerization of nucleoside diphosphates into a polyribonucleotide chain (RNA), and the reverse reaction by phosphorolytic degradation releasing also nucleoside diphosphates. In the case of in vitro RNA polymerizations catalyzed by PNPase, polyA and polyU prime their own synthesis (Grunberg-Manago et al. 1956), while polyC is a universal primer that primes its own synthesis and that of all polyribonucleotides (Mii and Ochoa 1957). However, the primer is not necessarily incorporated into the chain (Chou et al. 1975). After synthesis, the ribopolymer remains attached to the enzyme by multiple subsites (Godefroy-Colburn and Grunberg-Manago 1972). Physiological polymerization reactions can also be catalyzed by PNPase. For instance, PNPase from *E. coli* and chloroplast can perform both polyadenylation and degradation of mRNA (Monhanty and Kuhsner 2000; Yehudai-Resheff et al. 2001).

Biosynthesis and degradation of polyribonucleotides

(a) Ribonucleotidyl transferase

$$(\text{ribonucleoside} - \text{P})_n + (\text{ribonucleoside} - \text{PPP}) \rightarrow (\text{ribonucleoside} - \text{P})_{n+1} + (\text{PPi})$$

(b) Polynucleotide phosphorylase

$$(\text{ribonucleoside} - \text{P})_n + \text{Pi} \rightleftarrows (\text{ribonucleoside} - \text{P})_{n-1} + (\text{ribonucleoside} - \text{PP})$$

Fatty acids

Fatty acids act as storage molecules, secondary metabolites, and components of bacterial and eukaryotic membrane phospholipids. Fatty acids biosynthesis proceeds by condensation of acyl (malonyl-CoA) groups by iterative steps catalyzed by fatty acids synthases. In bacteria, acetyl-CoA is converted into malonyl-CoA by the acetyl-CoA carboxylase. A phosphopantetheine group from CoA is attached by an acyl-carrier protein synthase (ACPS) to the apo-ACP, and the malonyl-CoA:ACP transacylase charges malonyl-CoA to holo-ACP. Then, malonyl-ACP condenses with acetyl-CoA by β-ketoacyl synthase to yield ketoacyl-ACP, an intermediate reduced by β-ketoacyl reductase, dehydrated by β-hydroxyacyl dehydratase, and reduced again by enoyl reductase. A subsequent extension with malonyl-ACP by β-ketoacyl synthases initiates the elongation cycle.

Recent phylogenomic analyses suggest that archaea and the LUCA possessed a complete fatty acids synthesis

pathway, but it was devoid of acyl-carrier protein processing machinery (Lombard et al. 2012).

Biosynthetic pathways of fatty acids

$$\text{Acetate} + \text{Acetyl-CoA} \rightarrow \text{malonyl-CoA} \rightarrow \text{malonyl-ACP}$$

$$C_{2n}\text{Acyl-ACP} + \text{malonyl-ACP} \rightarrow C_{2n+2}\text{Acyl-ACP} + CO_2$$

Polyisoprenoids

Derivates of isoprenoids constitute the largest group of biological compounds, encompassing around 30,000 known products. They fulfill diverse biochemical functions as major structural membrane components in archaea, photosynthetic pigments, plant hormones, plant defence compounds and quinones acting in electron transport chains (Lange et al. 2000).

Polyisoprenoids are oligomers synthesized ubiquitously among eubacteria, archaebacteria and eukaryotes through condensations of the five-carbon compound isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP). Two independent non-homologous metabolic pathways are known: the mevalonate (MVA) pathway is present in eukaryotes and archaea while the methylerythritol phosphate (MEP) pathway occurs in bacteria and several photosynthetic eukaryotes. The MVA pathway is responsible for the production of IPP and DMAPP from two acetyl-coenzyme A (CoA) condensed by acetoacetyl-CoA thiolase. The MEP pathway is capable of generating IPP and DMAPP from D-glyceraldehyde-3-phosphate (G3P) and pyruvate. The MVA pathway is likely an ancestral metabolic route present in the three domains of life (Lombard and Moreira 2011).

Biosynthetic pathways of isoprenoids

(a) MVA pathway

$$2\ \text{Acetyl-CoA} \rightarrow \text{isopentenyl pyrophosphate/dimethylallyl pyrophosphate}$$

(b) MEP pathway

$$\text{Pyruvate} + \text{glyceraldehyde-3P} \rightarrow \text{isopentenyl pyrophosphate/dimethylallyl pyrophosphate}$$

Polypeptides

There are three natural microbial polypeptides synthesized by enzymatic processes in a template-independent way from the translational machinery. Two of them are extracellular, poly-γ-glutamate and ε-poly-L-lysine, and one intracellular called cyanophycin. Cyanophycin is a polyamide of L-arginyl-poly(L-aspartate) which proposed structure is a poly-α-aspartic acid backbone with arginine residues linked to almost all β-carboxyl groups by isopeptide bonds. It accumulates under stress conditions as insoluble inclusions in cyanobacteria. Apart from serving as reserve of nitrogen, it may play a more dynamic role integrating also carbon metabolism. Synthesis of cyanophycin is catalyzed by cyanophycin synthase and the initiation step in vitro requires a primer either $(\text{Asp-Arg})_3$ or $(\text{Asp-Arg})_2$. The elongation proceeds from the C-terminal of the poly-aspartate with the addition of aspartate followed by arginine. ATP provides the energy for peptide bond formation. Cyanophycin is depolymerized by cyanophycinase from its C-terminus releasing the dipeptide Asp-Arg (Oppermann-Sanio and Steinbüchel 2002; Stubbe et al. 2005).

Poly-γ-glutamate (10–1000 kDa) is a component of the cell capsule. The proposed synthetic reaction is catalyzed by a membrane protein complex and resembles an ATP-dependent (ADP-forming) amide ligase mechanism with an oligo-(γ-glutamate) phosphorylated at its carboxyl terminus followed by the addition of a glutamate residue. However, a non-ribosomal peptide synthetase (NRPS) mechanism was also proposed. A γ-glutamyl hydrolase might degrade the polymer (Hamano et al. 2013).

Poly-L-lysine is a linear homopolymer of 25–35 lysine residues with linkages between the α-carboxyl groups and the ε-amino groups. Polylysine synthase contains domains characteristic of NRPS. Intracellular ATP levels regulate its catalytic function. A reaction mechanism similar to amino acid ligase was proposed, but without phosphorylation of the C-terminus of the growing chain. L-Lysine molecules polymerize via multiple enzymatic reactions: adenylation, thiolation, and peptide formations. It was identified a polylysine degrading enzyme with endo-type peptidase activity (Hamano et al. 2013).

Multimodular megaenzymes NRPS—also by nucleic acid-independent synthesis—produce an enormous diversity of dipeptides, homo and heteropeptides decorated by assorted modifying enzymes, including glycosyltransferases, carbamoyltransferases, and oxidases (Finking and Marahiel 2004). The biosynthesis of certain peptide antibiotics such as gramicidin S and tyrocidin requires a thioester precursor (4-phosphopantetheine). On an earlier proposal, Lipmann (1971) suggested that this mechanism of peptide synthesis might have preceded ribosome-synthesized proteins in the origin of life. Recent phylogenomic analyses seem to support the idea that the non-ribosomal peptide synthetases and other peptide ligases would have appeared before the emergence of processive ribosomal protein synthesis (Caetano-Anollés et al. 2012).

Even in the case of a theoretical model, it is worth mentioning here that de Duve (2003) influenced by Lipmann's work, supported the assembly as "multimers" (to

distinguish them from true peptides) of hypothetical heterogeneous non-coded peptide-like compounds synthesized with the help of the energy derived from the thioester bond (thioester world). The multimerization would have yielded under different physical and chemical constraints a population of uncoded peptides containing amino acids of both L and D chirality and other bifunctional acids with catalytic activities involved in: electron transfer, phosphorylation, group transfer and energy coupling that allowed the establishment of a protometabolic network. Short multimers could have adopted molecular configurations similar to those of enzyme active sites. Moreover, in fact many multimers are cofactors that work together with modern enzymes enhancing their catalytic abilities.

Cofactors (chimeromers)

Most of the reactions in the metabolic chart require enzymes functioning in conjunction with cofactors. These compounds appear to be a distinct class of molecules called "chimeromers" by Srinivasan and Morowitz (2009) to reflect their distinctive constitution. They may contain some amino acids, some sugars, some sulfhydryl and heterocyclic nitrogenous groups. Major functional roles include electron transfer (glutathione, NAD, NADP, FMN, quinones), and group transfer (ATP, phosphate; SAM, methyl; nucleotide sugars, monosaccharides; pyridoxal, amino and carboxyl; coenzyme A, acetyl and acyl). In extant pathways of cofactor biosynthesis, each cofactor requires the involvement of other(s) cofactor(s) (Srinivasan and Morowitz 2009). This autocatalytic process supporting their own synthesis suggests that cofactors would have been ancient chemical operators. Cofactors would have functioned in intimate cooperation with peptides in a peptide–cofactor world (Raffaelli 2011).

Degradosome complex: connections among RNA, carbohydrate, polyphosphate and central carbon metabolisms

The principal constituents of the bacterial degradosome, a macromolecular complex involved in RNA processing and degradation (Rauhut and Klug 1999; Burger et al. 2011) are the RNAse E, the exoribonuclease PNPase, a DEAD box RNA helicase, a polyphosphate kinase (PPK) and enolase. The genes coding for the degradosome components and those involved in the synthesis and interaction with ribonucleotides and sugars are among the most conserved gene sequences in the three domains of life (Delaye et al. 2005).

The functional role of enolase in the degradosome is unknown. Enolase is an enzyme of the lower portion of the Embden–Meyerhof–Parnas and the Entner–Doudoroff glycolytic pathways that catalyzes the reversible and stereo-selective dehydration of 2-phosphoglycerate (chiral) to phosphoenolpyruvate (not chiral). It is involved in both glycolysis and gluconeogenesis reactions. Interestingly, yeast enolase binds nucleic acid (Al-Giery and Brewer 1992) while the mammalian one has RNA-binding activity (Hernández-Pérez et al. 2011). These activities suggest that enolase might be involved in RNA metabolism reflecting ancient connections between polysaccharides and RNA (bio)chemistries.

The degradosome complex in the *Caulobacter crescentus*, a Gram-negative α-proteo-bacterium contains the aconitase enzyme, instead of enolase (Hardwick et al. 2011). Aconitase is an enzyme of the Krebs citric acid cycle the core of the metabolic chart.

Polyphosphate kinase is also a component of the *E. coli* RNA degradosome (Blum et al. 1997). PPK catalyzes the polymerization of the terminal phosphate of ATP into a polyphosphate chain (polyP). PPK has RNA-binding activity and can bind and remove polyP, an inhibitory of RNA degradation by the degradosome (Blum et al. 1997).

Abiotic synthesis of polymers

So far, not all the possible abiotic polymerization processes have been explored and their relevance in primitive metabolism remains unknown. Moreover, the similarities between abiotic reactions and their enzyme-mediated counterparts in biosynthetic pathways do not necessarily indicate an evolutionary continuity between prebiotic chemistry and biochemical pathways. Here, the list of examples represents a rather limited set of plausible instances of non-enzymatic chemistry performed in anhydrous conditions or in aqueous solutions in contact with mineral surfaces.

Phosphate chemistry

Phosphates are ubiquitous in the biochemistry of living beings. However, the prebiotic source of a soluble form of phosphate remains controversial. A mechanism proposes that abiotic polyphosphates could have accumulated by the condensation of phosphates into pyrophosphate and triphosphate from the mineral apatite (calcium phosphate) exposed to hot basaltic magma (Yamagata et al. 1991; Schwartz 2006).

It was recently proposed that the phosphide mineral schreibersite $(Fe, Ni)_3P$ can react with water to form phosphate compounds with a multitude of oxidation states including phosphite, pyrophosphate, orthophosphate, triphosphate and phosphonates. Phosphite could be

relevant to prebiotic chemistry because it is significantly more soluble and reactive than orthophosphate. In the biosynthetic pathways, phosphite reacts with organic compounds to form phosphonates with a C–P bond. Phosphonates are present in many organisms from bacteria to mammalian, and lipids and cell membranes are the major reserve (Pasek 2008). Interestingly, meteorites may have introduced both reactive phosphates and phosphides species on the early Earth (Pasek 2008). Alternatively, the pyrophosphate (PPi) groups seem to be the only phosphate material found as a condensed abiotically mineral in canaphite, $CaNa_2P_2O_7{\cdot}4H_2O$ (Rouse et al. 1988).

Polyribonucleotides

Under abiotic scenarios, nucleoside 5′-polyphosphates synthesized in a plausible route from nucleoside 5′-monophosphates and inorganic high-energy condensed trimetaphosphate. Trimetaphosphate could be provided by depolymerization of linear inorganic polyphosphates in presence of Mg ions. Nucleoside 5′-polyphosphates could act as activated forms of nucleotides from which nucleoside 5′-mono-, di- and triphosphates would have accumulated (Lohrmann 1975, 1976).

Oligonucleotides up to six nucleotides long can be synthesized from cyclic 2′,3′-AMP when it was dried and heated (Verlander and Orgel 1974). Formation of RNA oligomers up to 50-mers occurs on clays that act as scaffolds to facilitate polymerization (Ferris et al. 1996).

PNPase synthesizes oligoribonucleotides from nucleoside 5′-diphosphates. It is known that the properties of PNPase to synthesize in vitro a polyribonucleotide chain with a composition proportional to the amounts of ribonucleoside diphosphates present were crucial to elucidate the genetic code. By calculating, the statistical composition of triplets in the heteropolymers allowed the groups of Ochoa and Nirenberg to establish the correspondence with most of the 20 amino acids (Nirenberg 2004). Even though the first polynucleotides would have been devoid of genetic information, it seems plausible to infer that the variations incorporated resulted in an uninformed complexity that became informational sequences later with the appearance of modular coding/decoding components.

Fatty acids

The Fischer–Tropsch chemical synthesis converts CO and hydrogen gases in the presence of transition-metal catalysts into liquid hydrocarbons. Under hydrothermal conditions hypothesized to exist in the Hadean, a similar process with formic acid or oxalic acid in stainless steel vessels produces lipid compounds such as *n*-alcohols, *n*-alkanoic acids, *n*-alkyl formates, *n*-alkanals, *n*-alkanones, *n*-alkanes, and *n*-alkenes (McCollom et al. 1999). The reductive acetyl-CoA pathway may be a biological equivalent of the Fischer–Tropsch process, in that CO builds up to hydrocarbon compounds (Fuchs 2011).

Sunlight, as energy source, has also been shown to drive the synthesis of long-chain hydrocarbon amphiphiles through the photochemical oxidation of alkanes in the presence of polycyclic aromatic hydrocarbons (reviewed by Volkov et al. 1995).

Both long-chain monocarboxylic acids and polycyclic aromatic hydrocarbons with amphiphilic properties were extracted from the Murchison meteorite which predates the formation of Earth, and able to form vesicles spontaneously in water. In addition, these molecules can absorb light and translocate protons across the bilayers providing possibilities of energy transduction by chemiosmotic mechanisms (Deamer and Harang 1990; Deamer 1997).

Polyisoprenoids

The isopentenyl units can be obtained industrially by acid catalyzed Prins reaction from isobutene and formaldehyde (Blomquist and Verdol 1955). Isobutene, ethylene and formaldehyde precursors occur in volcanic gases, suggesting a plausible prebiotic synthesis. The condensation reaction of isopentenyl units might have proceeded as the head-to-tail elongation by anchoring one end of the molecule to a solid mineral surface through a phosphate group (Ourisson and Nakatani 1994).

Peptides

A number of experimental models tested condensation reactions of amino acids to form peptides. The first proposals used thermal copolymerization of amino acids to form protein-like chains through the formation of peptide bonds at temperature over 140° or at 70 °C in the presence of phosphoric acid under hypohydrous conditions (Fox and Harada 1958). Proteinoids rich in lysine are able to synthesize offspring peptides. Lysine-rich proteinoids also catalyze the polymerization of nucleoside triphosphate to polynucleotides and assemble to cell-like microstructures (Fox 1981).

Synthesis and degradation of peptides under geological relevant conditions also occur with activated amino acids with CO and H_2S in the presence of coprecipitated (FE, Ni)S and Na_2S suggesting a possible primordial peptide cycle (Huber and Wächtershäuser 1998; Huber et al. 2003).

Plausible as prebiotic reaction, carbonyl sulfide volcanic gas used as condensing agent promotes the formation of peptides under mild conditions in aqueous solution. The peptide condensation is stable toward hydrolysis and

enhanced in the presence of Pb^{2+}, Fe^{2+}, or Cd^{2+} metal ions, oxidizing agents, or alkylating agents (Leman et al. 2004).

The divalent copper ion catalyzes in the presence of high NaCl concentration as strong dehydrating agent, the salt-induced peptide formation reaction at temperatures between 60 and 90 °C. It represents the simplest way to obtain peptides in aqueous solutions compatible with conditions presumably present in the primordial environment. The reaction works with all amino acids and shows preference for the biologically relevant L-form of α-amino acids over β- and γ-amino acids (Jakschitz and Rode 2012).

Cofactors

Pantetheine and nicotinamide precursors of CoA and NAD cofactors can be synthesized under simulating early chemical conditions. For example, pantetheine is synthesized by heating pantoyl lactone, β-alanine and cysteamine at temperature as low as 40 °C (Keefe et al. 1995), while nicotinamide is easily synthesized non-enzymatically from dihydroxyacetone phosphate and aspartate (Cleaves and Miller 2001).

Many cofactors in modern metabolism are riboadenosyl derivatives (NAD, NADP, FAD, coenzyme A, ATP, etc.) or contain heterocyclic nitrogenous groups (thiamin pyrophosphate, tetrahydrofolate, pyridoxal phosphate, etc.). Formation of the linkages in some coenzymes can be catalyzed by ribozymes using in vitro selection (Jadhav and Yarus 2002). The RNA-world could derive from improvements in cofactor chemistry (Caetano-Anollés et al. 2012).

Notions of organizational closure and emergence

Living systems are thermodynamically open systems in which energy and matter may transfer into or out of them from the environment. Maintenance of life depends on both a high degree of internal order (self-assembly) and far-from-equilibrium reactions (self-organization). Self-assembly (Deamer 2011) is an autonomous (without external intervention) closer-to-equilibrium process that results in the aggregation of molecular components into large structures through weak interactions (medium-range electrostatic forces, van der Waals forces, but non-covalent bonds), and only possible at the expense of an entropy increase in the surroundings. Self-organization (Ruiz-Mirazo 2013) involves far-from-equilibrium catalytic reactions (dissipative conditions), where exergonic processes (spontaneous, which release energy) coupled with endergonic ones (non-spontaneous, which requires energy). Living systems maintain themselves far from thermodynamic and chemical equilibrium performing work with the energy obtained from the metabolic pathways, whereas nonliving systems tend spontaneously to develop toward thermodynamic and chemical equilibrium.

Another essential property of livings systems that makes them different from physical systems is that organisms constitute an organizational closure, a distinctive circular causal regime. The metabolic reactions are organized in a closed pattern. Each constituent is catalyzed by another constituent. The components are recursively regenerated by the network (self-reproduction), and all components contribute to maintaining themselves by mutual dependence (self-maintenance). The organization is product of itself (self-organization). No external activities are needed for maintaining the system. (Letelier et al. 2011; Mossio and Moreno 2010; Ruiz-Mirazo 2013). Living systems maintain the organizational closure producing their own physical boundaries from the ambient and internal environments by compartmentalization through membranes. Compartmentalization prevents dispersion of metabolic intermediates, facilitates molecular reactions by means of confinement and separates reactions that might conflict with one another.

The organization closure can also be taken as a higher-level emergent configuration with specific relations and interactions among its own constituents. The configuration is irreducible to the intrinsic properties of its constituents and non-derivable from the knowledge of the components. The organization closure generates functional properties that only the whole system possesses whereas the parts taken separately do not exhibit (emergent regime of causation); the whole organization realizes self-maintenance (Mossio et al. 2013). These authors also argue that an emergent closed organization does not necessarily imply inter-level causation (upward or downward causation) between the whole and its own parts.

Wicken (1987) combining physicalism and organizational relationships defines life as a system that maintains a high degree of internal order by dissipating entropy to its surroundings. Contrary to conventional wisdom of the second law of thermodynamics as disorganizing principle, he claimed that the second law in fact acts to dissipate order (entropic production), not organization. The emergence of life is a consequence of the driving force of the second law: the necessity of the prebiosphere to discharge energy gradients in self-maintaining and self-proliferative dissipative processes to create entropy. The second law depletes thermodynamic potentials and promotes the biological evolution by formation of structures of growing complexity. Life as a thermodynamic system has a material structure and a finite set of kinetic possibilities within this structure. The physical world includes a dynamics of self-

organization. The emergence of life falls fully within the realm of physical nature (Fry 1995; Egel 2012).

However, the principles that govern the emergence remain a subject of intense debate and criticisms. For example, Kim (2006) known for his work on mental causation and the mind–body problem claims that classic emergentism denies the deductibility of the behavior of a whole from the properties of its constituents. However, emergent properties strive to be compatible with a given appropriate basal condition from which they will emerge (emergents supervene on their base properties, supervenience), and to influence events and processes at lower-level (downward causation). In his view, it means that emergent properties (supposed to be novel) will be reducible to physical causes and hence nonemergent. But as has been noted by Mossio et al. (2013), the central idea is that the relevant supervenience base is not a set of properties of constituents taken individually, but rather the properties of the configuration, the whole set of inherent and relational properties of the constituents.

Models of organization closure

According to the properties mentioned above, most theories of the origin of life and their respective models gravitate around the notion of closure, the idea of living systems as self-constructing systems (Letelier et al. 2011; Mossio 2013). For example, the hypercycle model (Eigen and Schuster 1978) is a closed arrangement of self-replicative cycles connected by catalytic reactions. In the reaction scheme of the "realistic" hypercycle, an information-coding RNA molecule (I_1) specifies its own reproduction and the structure of an enzyme (E_2), which in turn catalyzes the replication of other different information molecule (I_2) to initiate the next cycle. The hypercycle projects into a tentative RNA-world hypothesis: the information-coding molecules should be simultaneously catalysts and replicators. They must have the ability to self-replicate and to have a catalytic function. Hypercycles attempt to extend Darwinian evolution to the origin of life. Accordingly, hypercycles by mutation and natural selection would build up all of the substances and ultimately the processes and structures of living cells. However, Wicken (1985) claimed that livings systems do not show any clues of hypercyclic ancestry. It is difficult to envision the emergence of a translation capacity from that system. How could such replicators begin making proteins? Wicken proposed that life emerged through the coevolution of nucleic acids and proteins within catalytic microspheres—a concept developed by Fox (1980) as a prerequisite for the emergence of translational machinery. He considered this approach "organismic" in the sense that the emergence of life was the result of a part-system relationship, instead of supposing that one constituent could be derived from the other.

The so-called (M, R) systems or "metabolism-repair" systems (Rosen 1991) refers to the idea that a living system requires continuous replacement of all its components to maintain it. This would leads to an infinite regress where one molecule needs other to synthesize another and so on. To solve this problem, Rosen appeals to Aristotelian categories of causality: the organisms are open to matter and energy (openness to material causation), but closed to external activities for organizing its own metabolism (closure to efficient causation). All of the properties determining the organization are generated from within the system itself. According to Rosen, the closure is realized by three broad biological functions: metabolism, repair and replication. The mathematical model expresses a circuit in which the components are mutually entailed (Mossio 2013; DiFrisco 2014).

The concept of catalytic closure in the autocatalytic sets of Kauffman (1993) focuses on what sort of spontaneous conditions might allow self-organization to arise from stochastic properties of sets of molecules, independently of Watson–Crick template replication. The model is composed of a set of organic molecules forming a reaction network; a set of input "food" organic molecules to drive the system away from chemical equilibrium; and a set of organic molecules within the reaction network such that each served as a catalyst to one or more reactions. The set of molecules collectively catalyzed all the last steps in the formation of each member. The studies of the model showed that as the diversity of molecules in the system increases, the diversity of reactions among them increases even faster and, more and more reactions are catalyzed leading to a network of reactions. Then, the entire set is able to catalyze its own production, and the system becomes collectively autocatalytic (Kauffman 2011).

The theory of autopoiesis (Maturana and Varela 1980) refers to a closed network of processes of production, transformation and destruction of components that recursively realize and regenerate the same processes and components, and that builds up its own physical border distinguishing itself from its environment. The autopoietic production of a boundary, such as a membrane, lies at the root of the autonomy of living systems. However, the autopoietic system is structurally coupled with the environment through dynamic exchanges of matter, energy and information. The organization of living systems as a closure deals with the reciprocal causation between the components and a network of relations that may obtain between them, but not with their properties which are appropriate to the phenomena of physics. The operational closure describes the system at the chemical and molecular level, and

supposes relations of material production among its constituents. The autopoietic system is a unit constituted by a global network of component relations that establish a self-maintaining dynamics (Mossio 2013; DiFrisco 2014). The autopoiesis theory refers to what is a living system, but it is not constructed as a theory concerning the origin of life, because in its initial conception the establishment of an autopoietic system is not a gradual process with intermediate states (Maturana and Varela 1980). However, the criteria of autopoiesis provide a fruitful conceptual framework for approaching the organization of living systems (Luisi 2003).

The chemoton (chemical fluid automaton) is a theoretical model for a protocell proposed by Ganti (2003). It describes a system at chemical level that embodied the minimal life criteria for a living organism. The life criteria include that the living system is a unity, performs metabolism to maintain non-equilibrium, is inherently stable, contains information about itself, and regulates and controls its own processes. The system is autocatalytic and composed of three stoichiometric interconnected reaction cycles: one metabolic-independent of enzymes that captures energy and produces the source materials, one that replicates molecular information, and one cell membrane for structural organization. Each component catalyzes the formation of the next compound in the reaction cycle. The integration of the different reaction cycles and the mutual dependence between them result in an organization that collectively ensures the self-maintenance. The model assumes self-reproduction by fission (growth in size and division), and self-maintenance without heritance variations through cycles that regenerate the components that are catalytic and created by the system itself. This causal regime constitutes a minimal organizational closure (Mossio and Moreno 2010), a crucial step in the transition from physicochemical to simple biological systems capable of increasing their complexity to become living organisms. Nevertheless, the chemoton model attempts to construct a living system by abstracting the general properties of a metabolic closure (chemical cycles and known chemical principles) without specific requirements for the nature or the composition of its chemical components. It remains an open question what compounds could be arranged in an appropriate way to account for a minimal organizational closure.

Deacon (2006) proposes an autocell model consisting of a reciprocal linkage between an autocatalytic cycle and self-assembling encapsulation process where the constituents (proteins) for the capsule are products of the autocatalysis. Autocell self-repair, self-reproduction and structural conservation are not dependent of membrane containment and do not need self-replicating template molecules. The emergence of autocellularity requires significant amount of large polymers sharing structural similarities and with sufficient variety and degeneracy of their stereochemical properties to support spontaneous autocatalysis and self-assembly. Deacon suggests a taxonomic classification of functional organization designated "*Autaea*" for all autonomous and self-supportive configurations of matter including living organisms and chemical systems. Within *Autaea*, the taxon *Morphota* is characterized by morphological reproduction, while *Semeota* is distinguished by reproduction via molecular coding. Autocells are members of *Morphota*, capable of leading to more complex forms. *Semeota* derives from *Morphota*, which due to their relative simplicity would have predominated at early stages.

Egel (2009) presumes a peptide-dominated scenario based on the autocatalytically closed sets of statistical peptides proposed by Kauffman (1993) preceding the RNA world. Prebiotic oligomeric components could confine by colloidal coherence in a hydrogel matrix as happens in modern protoplasm. Egel also provided a detailed sequence of reaction pathways and structural confinement steps integrated into closed systems from protometabolism to protocells. More recently, Egel (2014) has proposed new perspectives on the earlier hypothesis of Sidney Fox (1980) about the boundary-independent proteinoid microspheres. Coevolution of uncoded catalytic peptides, cofactors and oligoribonucleotides would have allowed a graded transition from proto-biofilms to protocells.

Phylogenomic studies suggest a complex scenario for the origin of life with an expanding set of associated chemistries and molecular interactions in a world of macromolecules: nucleic acids, proteins, cofactors, lipid membranes and other components that supported a gradual rising of cellular organization (Caetano-Anollés and Seufferheld 2013). The model proposes that early protocells used amphiphiles with fatty acids chains and other molecules embedded in the membranous structure (peptides, polyphosphate, polyhydroxybutyrate) that could enhance the growth and persistence of the emerging protocells. Short peptides similar to natural peptide antibiotics inserted onto the expanding membranes would gain catalytic function and act as Kauffman's peptides: dipeptidase and peptide ligation activities, carrier and channel-forming properties and ability to bind to numerous cofactors (Caetano-Anollés et al. 2012).

Nontemplate-driven polymers: proposed minimal form of organizational closure

As mentioned above, contemporary polyribonucleotides, polysaccharides, polyhydroxyalkanoates, peptides, polyphosphates, fatty acids and polyisoprenoids can be

synthesized and degraded by enzyme-catalyzed nontemplate-dependent polymerizations. Moreover, many of these polymers presumed to have existed in prebiotic environments were prepared in abiotic conditions. In this sense, it seems reasonable to expect that primordial polymers would have emerged by nontemplate-driven polymerization from intertwined chemical reactions sharing basic compounds obtained from environmental inputs.

Borrowing from metabolism-first models the independence of template replication and including the notions of openness to matter and energy, closure to external activities, containments or phase-separated systems and within a world of macromolecules, it is plausible to envisage a minimal organization closure putting in the role of the abstract components nontemplate-dependent polymers and their units.

In the context of the precursory metabolic networks (Orgel 2008; Egel 2011), it would not be unreasonable to conjecture that the nontemplate-driven polymers could have organized in two distinct polymerization cycles as the result of two distinctive reaction pathways; one of them involving oxo acids and acetyl/acyl CoAs, the other requiring phosphorylated intermediates. A first polymer reaction cycle would posit on compounds obtained from a reductive carbon assimilation pathway relying on acidic moieties provided by carboxylic groups and thioesters as the energetic bond. The polymers of this cycle comprise polypeptides, polyhydroxyalkanoates, fatty acids and polyisoprenoids. The other cycle of polymerization reactions would involve sugar phosphate metabolites. These metabolites would originate from the pentose phosphate/glucogenesis pathways and/or Calvin cycle with acidity and energy provided by phosphoric bonds. This cycle consists of polysaccharides, polyphosphate and polyribonucleotides (Freire 2011). Pyruvate might have connected both metabolic reaction cycles.

Overlapping reaction pathways, interconnected and reversible polymerizations, multifunctional constituents and phase-separated systems (fatty acids, proteinoid microspheres, peptide vesicles or hydrogel matrix) might have provided selective advantages: allow recycling and sharing of components, proofreading, saving energy activities and functionally specifiable contributions to the self-maintenance of an organizational closure (Fig. 1).

Polyribonucleotides as repository would have served a dual role providing energy and building blocks ribonucleoside diphosphates in the synthesis of polyP, or combined with sugars as glycosyl donors in the synthesis of polysaccharides. Polyribonucleotides would have gradually assumed a new role in the transmission of information as template (Mirsky 1959). Polysaccharides would have shared sugar units with polyribonucleotides. Polysaccharides also acted as cleaners and concentrating agents (hydrogel matrix) in the synthetic reactions of other polymers under physicochemical constraints (Tolstoguzov 2004), and scaffold for the assembly of other macromolecules (Stern and Jedrzejas 2008). Phosphoric acid and polyphosphates may have served as the prebiotic source for phosphorylation reactions. For example, sugars are universally phosphorylated, this further enhanced their solubility and reactivity (Srinivasan and Morowitz 2009). PolyP might have acted as energy donor, providing pyrophosphate (PPi) or eventually nucleoside diphosphates/triphosphates used as cofactors in the synthesis of polysaccharides and polyribonucleotides, and phosphate (Pi) for their phosphorolytic degradation (Freire 2011). PolyP might have served as chaperone binding unfolded peptides (Gray et al. 2014) and scaffold for the synthesis of other polymers (Kornberg 1995; Schramm et al. 1962).

Polyhydroxyalkanoates as reserve of carbon and energy (Stubbe et al. 2005) would have provided acetate and/or hydrocarbon chains for the synthesis of fatty acids, polyisoprenoids and peptides. Peptides apart from serving as reserve of nitrogen, they could have catalyzed elementary reactions. For example, proteinoids rich in lysine catalyze the polymerization of peptides and polynucleotides, and assemble to cell-like microstructures under the hydrophobic tension of an aqueous environment (Fox 1981). Within a late onset of cellular individualization, peptide-dominated vesicles together with isoprenoid pigments could engage in light harvesting before the emergence of external boundary (Egel 2009).

Amphiphilic peptides with hydrophobic tails and hydrophilic heads could also self-assemble into micelles, nanotubes and nanovesicles with stability in water. These lipid-like peptides could form simple enclosures that stabilized other molecules, modified local concentration and favored the dehydration process for chemical bond formation (Zhang 2012).

Dipeptide seryl-histidine and related oligopeptides cleave DNA, proteins and carboxyl ester (Li et al. 2000), and catalyze the formation of peptides and peptide nucleic acids (Gorlero et al. 2009).

Early uncoded peptides might have also performed a variety of chemically functions such as anion and cation binding and membrane and channel formation as well as simple catalysts through distinguished motifs. For example, short polypeptides rich in glycine residues tend to form anion-binding nest structures which have affinity for iron-sulfur clusters and phosphate groups (Milner-White and Russell 2005). The most common cation-binding motif is the niche. Three functionally useful cation-binding features are: calcium binding; sodium and potassium ion transport across membranes in peptide channels; and covalent metal-peptide complexes where the peptide binds copper, nickel, cobalt or iron metal ions, for catalysis (Milner-White and Russell 2011).

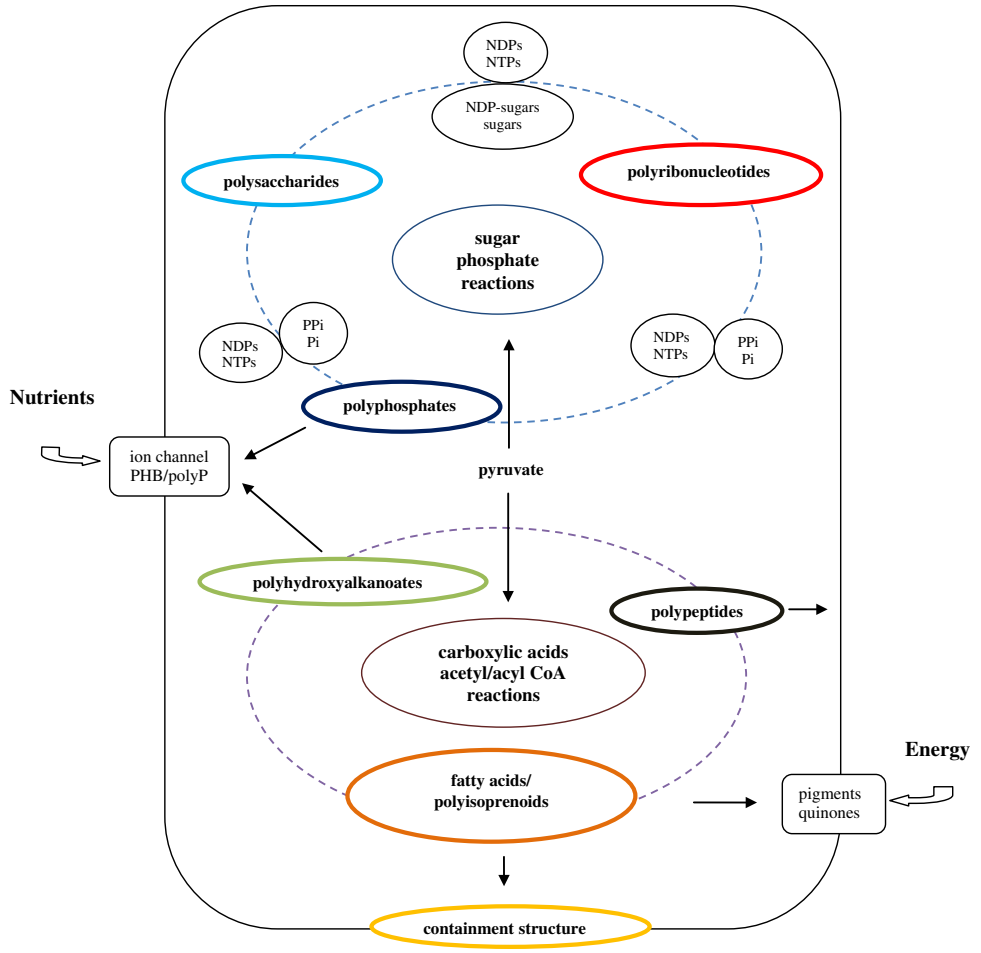

Fig. 1 Nontemplate-driven polymers: metabolic connections and organizational closure. Synthesis and degradation of polysaccharide, polyribonucleotide and polyphosphate polymers would have shared chemical reaction pathways, activated compounds and cofactors obtained from a related pentose phosphate/gluconeogenesis and/or Calvin cycle: sugars, sugar phosphates, NDP (ribonucleoside diphosphate), NDP-sugars, NTP (nucleoside triphosphate), PPi (pyrophosphate) and Pi (inorganic phosphate). Polyhydroxyalkanoates, fatty acids/isoprenoids and simple polypeptides might have been formed from a carbon assimilation pathway that provided compounds like carboxylic acids and acetyl/acyl CoA. Peptides and/or self- assembly amphiphiles would have contributed to the emergence of containment structures. Polyhydroxyalkanoate/polyP complexes allowed the formation of ion channels, and isoprenoids provided compounds to harvest energy. Peptides could have limited catalytic repertoires

Polyphosphates might have reacted with amino acids chemically causing the formation of peptides, while the peptides probably mediated by nest motifs might have catalyzed the formation of polyphosphates. This mutual synergy would have been a key feature at early stage in evolution before the advent of the ribosomal peptide synthesis (Milner-White and Russell 2010).

PolyP would have formed volutin-like granules (Kornberg 1995) and together with fatty acids might have contributed to the emergence of self-assembled compartments establishing boundaries with the environment and encapsulating chemical reactions. Polyhydroxyalkanoates/polyP complexes would have allowed the formation of ion channels, increasing the selectivity of chemical reactions, taken advantage of potential energy of ion gradients across the membranes (Reusch 1999). The generation of proton gradients could drive the formation of polyphosphate from inorganic phosphate, which could in turn have been used as chemical energy source to drive other reactions, including polymerization of amino acids to form polypeptides (Weber 2000). Weakly catalytic peptides and polyphosphate could have helped the polymerization of nucleoside phosphates into polynucleotides (Weber 2000).

Gelling properties of polysaccharides could have increased the local concentration of chemically different proto-biopolymers, fixed their relative positions, improved their chemical stability against hydrolysis and favored their interactions. Attractive–repulsive interactions between polymers could develop novel emergent activities (Tolstoguzov 2004). For example, the close proximity of

polypeptides and polynucleotides within the protocellular space would have increased the likelihood of stabilizing interactions through ribonucleic acid-protein complexes, which later on developed into RNP machines for RNA-encoded protein synthesis (Weber 2000).

Wicken (1985, 1989) based on experimental evidences obtained by Fox and co-workers, suggests that catalytic microspheres might provide the organizational context for the emergence of the genetic code and the translation system. Proteinoids and nucleic acids established synergistic relationships and formed mutually stabilizing complexes. Selectivities between amino acids and nucleotides in these complexes could have allowed the emergence of the genetic code. In addition, microsphere regarded as model ribosomes in both structure and function allowed the emergence of relevant properties to carry out certain protometabolic activities.

A structural model (Carter and Kraut 1974) proposes that a polypeptide double helix would be precisely complementary to double-stranded RNA. A polypeptide double helix fits into the minor groove of the RNA double helix through hydrogen bonds between the ribose 2′ OH groups and carbonyl oxygen atoms. Adjacent nucleotide and peptide strands are parallel (5′–3′; N–C) and the two hybrid sets are antiparallel. In this complex, the RNA and polypeptide double helixes would have reciprocal templating polymerase activity. The RNA catalyzed condensations of polypeptides from some activated monomers, while the polypeptide catalyzed condensations of polynucleotide from other precursors. A structural complementarity of peptides and RNA is present in the modern ribonucleoprotein complexes of aminoacyl-tRNA synthases with their cognate transfer RNAs. In this sense, experimental evidences show that Urzymes, which are small catalysts derived from invariant core of aminoacyl-tRNA synthases and probably present much earlier than LUCA, catalyze amino acid activation, acylate tRNA and reduce the kinetic barrier to protein synthesis in the absence of ribosomes, suggesting that translation could begin in a peptide-RNA world (Li et al. 2013).

Peptides, polyphosphates and polyhydroxybutyrates could have facilitated the growth and persistence of protocellular structures. Peptides embedded in the membranous structures could have enhanced the growth and membrane stability of protocells upon environment fluctuations (Caetano-Anollés et al. 2012). Polyisoprenoids could have served as a reserve of isopentenyl pyrophosphate that would have acted as a primitive source of energy and provided a plethora of compounds such as pigments and quinones capable of harvesting radiant energy or redox potential.

Concluding remarks

The origin of life seems to have been the result of a long process of emergence rather that an event or sequences of discrete events in which many different possibilities were tested. Although it is unknown if compartmentalization was an early or a relatively late event, primitive chemical systems could have constituted minimal organization closures integrating simple, but different components in a sort of mutual dependence that would give rise to collective self-maintaining dynamics. The minimal organization closure sketched here would have morphological reproduction and included within the taxon *Morfota* that does not qualify as a genuine alive system according to Deacon (2006). However, the organization would allow the transition from the physicochemical to the biological domain. The increase of functional complexity to full-fledged organisms would achieve by incorporating template-dependent replication and gene-encoded protein synthesis later on.

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