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ORIGINAL PAPER

Polymer phosphorylases: clues to the emergence of non-replicative and replicative polymers

Miguel Angel Freire

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Abstract Polymer formation is arguably one of the essential factors that allowed the emergence, stabilisation and spread of life on Earth. Consequently, studies concerning biopolymers could shed light on the origins of life itself. Of particular interest are RNA and polysaccharide polymers, the archetypes of the contrasting proposed evolutionary scenarios and their respective polymerases. Nucleic acid polymerases were hypothesised, before their discovery, to have a functional similarity with glycogen phosphorylase. Further identification and characterisation of nucleic acid polymerases; particularly of polynucleotide phosphorylase (PNPase), provided experimental evidence for the initial premise. Once discovered, frequent similarities were found between PNPase and glycogen phosphorylase, in terms of catalytic features and biochemical properties. As a result, PNPase was seen as a model of primitive polymerase and used in laboratory precellular systems. Paradoxically, however, these similarities were not sufficient as an argument in favour of an ancestral common polymerisation mechanism prior to polysaccharides and polyribonucleotides. Here we present an overview of the common features shared by polymer phosphorylases, with new proposals for the emergence of polysaccharide and RNA polymers.

Keywords Degradosome · Enolase · Polyphosphate · Pyridoxal phosphate · Origin of life

M. A. Freire (✉)
Instituto Multidisciplinario de Biología Vegetal. CONICET. Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Av. Vélez Sarsfield 299, segundo piso, 5000 Córdoba, Argentina
e-mail: mig.frei@yahoo.com.ar

Introduction

Living organisms possess two types of polymers that are synthesised by template-dependent and non-template-dependent processes. One of the central problems on the origin of life concerns the mechanisms by which monomers could have been linked into biologically relevant polymers, as well as the manner in which these molecules were integrated into organised systems.

Derived from the central dogma, we know that DNA, RNA and protein polymers constitute a copying, coding and decoding information system. The self-maintenance and transmission of the information (heredity) is ensured through template-dependent polymerisation reactions. The remaining biopolymers and all other biological compounds are synthesised by non-template-dependent processes and do not contain a mechanism for heredity.

Most existing hypotheses on the origin of life assume that either the replication-first (genetic-first) or metabolism-first scenario have ancestral primacy. Modern nucleic acids and polysaccharides are the archetypes of these opposite scenarios where the former are synthesised by template-dependent replicative processes and the latter are products of non-template-dependent polymerisation reactions. However, this distinction becomes less clear when exploring the diversity of RNAs and polysaccharide molecules as well as the plethora of enzymes capable of their synthesis. Most of the template-independent RNA polymerases belong to the pol β superfamily of nucleotidyltransferases. 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). Curiously, polynucleotide phosphorylase (PNPase), another

nucleotidyltransferase but with distinct scaffolds, is capable of template-free RNA synthesis.

Brief history of polynucleotide phosphorylase

The discovery of nucleic acid polymerases in the second half of the 1950s, by the research groups of Severo Ochoa and Arthur Kornberg (both formerly at the Cori laboratory), appears to be due to more glycogen metabolism than to the structure model of DNA proposed earlier by Watson and Crick (1953a). Kornberg in particular, stated that "Glycogen phosphorylase, not base pairing, was what led me to DNA polymerase. (…) I had in mind an enzyme that, like the Cori glycogen phosphorylase, would extend a DNA chain by successive additions of a properly activated nucleotide" (Kornberg 2001).

The working model of in vitro synthesis of glycogen by glycogen phosphorylase was exceptionally useful in the identification of nucleic acid polymerases. Part of the success could be explained by an inherent biological fact: glycogen phosphorylases and nucleic acid polymerases and their respective polymers share essential properties. This is clearly reflected by the discovery of polynucleotide phosphorylase. PNPase was the first enzyme with a nucleic acid polymerase activity to be identified (Grunberg-Manago and Ochoa 1955), and with the similarities to glycogen phosphorylase, it was named a phosphorylase rather than RNA synthase.

Glycogen phosphorylases, belonging to the large family of glycosyltransferases, occur in the three domains of life (Archaea, Bacteria and Eucarya) and catalyse the phosphorolysis of α-glucan substrates from bacteria (maltodextrins), plant (starch) and animals (glycogen). PNPase catalyses the reversible polymerisation of ribonucleoside diphosphates, with the release of inorganic orthophosphate. It has a 5′–3′ RNA polymerase activity and is a 3′–5′ exoribonuclease that generates ribonucleoside diphosphate when degrading RNA.

Catalytic reaction of PNPase

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

Catalytic reaction of glycogen phosphorylase

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

From in vitro RNA polymerisation reactions to the replicative model

In 1939, Cori and Cori, showed that the in vitro synthesis of glycogen by glycogen phosphorylase prepared from distinct tissues of liver had a lag period, and that it was possible to abolish this period by adding small amounts of glycogen.

A lag phase was also observed prior to the synthesis of polyribonucleotides by PNPase, primed by polyribonucleotides with a certain degree of specificity. For example, poly-A and -U only primed their own synthesis while poly AU primed the synthesis of both polyribonucleotides (Grunberg-Manago et al. 1956).

These properties indicated the operation of a template-dependent mechanism for the formation of RNA with a specific sequence of nucleotides, in line with the replicative model. However, it was not apparent how PNPase could establish a specific nucleotide sequence of RNA because it seemed to randomly polymerise nucleoside diphosphate, independently of a template.

From the replicative model to RNA degradation

The discovery of DNA-dependent DNA polymerase activity by Kornberg et al. (1956) encouraged different laboratories to search for DNA-dependent RNA polymerases, reported in the early 1960s by several laboratories. Both DNA and RNA polymerases are template-dependent and use nucleotide triphosphates as substrate. In contrast, PNPase does not require a template, cannot copy one, uses ribonucleoside diphosphates and does not show specificity for bases. All these properties led to the conclusion that PNPase was not a replicative enzyme. Moreover, under physiological conditions and in view of its mechanistic properties and the relatively high phosphate concentration in the cell, PNPase was assumed to participate in RNA degradation and to be unable to synthesise RNA in vivo, in spite of its in vitro synthesis.

Similarly, until the 1950s, based on in vitro synthesis of glycogen, glycogen phosphorylase was believed to be capable of catalysing both synthesis and breakdown of glycogen. Further in vivo studies led to the idea that glycogen phosphorylase might be implicated in breakdown reactions while the synthesis of glycogen involved another enzyme. The synthesis of glycogen from UDP-glucose by glycogen synthase was characterised at the end of the 1950s by Leloir and co-workers (Leloir 1983).

Enzymatic mechanism

Both glycogen phosphorylase and PNPase enzymes have phosphorolytic activity but the reactions they catalyse yield different bonds. The reaction catalysed by glycogen phosphorylase leads to the formation of phosphoacetate in glycosyl 1-phosphate, while the one catalysed by PNPase produces pyrophosphate bonds in nucleoside diphosphates

(Cohn 1961). PNPase has since been classified as a phosphate-dependent 3′ exoribonuclease that catalyses the degradation of polyribonucleotides by phosphorolytic cleavage of the phosphodiester bond (Zuo and Deutscher 2001).

Both polymerisation and phosphorolysis proceed through a non-synchronous mechanism referred to as processive, where the enzyme completes the placement/displacement of nucleotide units one-by-one, without dissociation of the polynucleotide, before transferring its action to the other chain. A processive mechanism was initially proposed for polysaccharide phosphorylase from amylase (Bailey and French 1957). The kinetic enzymatic reaction catalysed by PNPase occurs through a rapid equilibrium, random Bi–Bi mechanism (Chou et al. 1975). A similar mechanism was earlier reported for polysaccharide phosphorylase for maltodextrin (Chao et al. 1969).

Energy capture

In contrast to hydrolytic nucleases, PNPase conserves the phosphate bond energy because it releases nucleoside diphosphates not monophosphates, reducing the energy demand for turnover of RNA.

Under physiological conditions, phosphorolysis of polynucleotides to yield nucleoside derivates is more exergonic than pyrophosphorolysis to yield nucleotide triphosphate (Stadtman 1973). Phosphorolytic cleavage of RNA is more suitable for degradative purposes than pyrophosphorolysis and allows the recapture of the phosphodiester bond energy, lowering the energy requirements.

In the case of glycogen phosphorylase, it has been pointed out that phosphorolysis preserves the energy of the glycosidic bond in sugar phosphate esters, which can be used in subsequent glycolytic pathways (Palm et al. 1990).

Biosynthesis of RNA and α-glucans by polymer phosphorylases

Physiological polymerisation reactions can be catalysed either by glycogen phosphorylase or PNPase. For instance, it has recently been reported that PNPase from *E. coli* and chloroplast can perform both polyadenylation and degradation of mRNA (Monhanty and Kuhsner 2000; Yehudai-Resheff et al. 2001). In the case of glycogen phosphorylase and glycogen synthase, crystallographic studies have showed a high level of topology and structural similarity, even though there is a reduced sequence identity (Buschiazzo et al. 2004). These observations hint that these two enzymes might share similar catalytic mechanisms and leaves open the possibility for in vivo synthesis of glycogen by glycogen phosphorylase.

Pyridoxal phosphate cofactor

Pyridoxal phosphate is a coenzyme with a nucleotidyl moiety thought to be a molecular fossil of prebiotic chemistry as a coribozyme (Jadhav and Yarus 2002).

Baranoswski et al. (1957) found that the pyridoxal phosphate cofactor was bound to rabbit muscle glycogen phoshorylase. The pyridoxal 5′ phosphate (PLP) is bound into the centre of the protein, behind the active site, with its phosphate group adjacent to the binding site of the phosphate of glucose-1P. It probably assists the attachment of a phosphate ion on the oxocarbonium formed by cleavage of the glycogen chain.

All α-glucan phosphorylases studied catalyse the phosphorolysis of an α-1-4 polyglucose chain, with the anomeric carbon configuration containing PLP, without which they are inactive. It has been hypothesised that PLP is not required for phosphorolysis itself but that it may be involved in the catalysis of this process (Graves and Wang 1972). In the case of glycogen synthetase, the ribose and the distal phosphate of ADP are at equivalent positions to the pyridoxal groups and inorganic phosphate on glycogen phosphorylase (Buschiazzo et al. 2004).

The exoribonuclease-phosphate dependent family members (PNPase and RNAse PH) contain a single highly conserved domain (PH) characterised by three conserved sequence motifs, where one is positively charged and may bind the phosphate substrate, thereby defining the exoribonuclease activity of this superfamily (Zuo and Deutscher 2001). Mamaeva et al. (1979) reported competitive inhibition of polynucleotide phosphorylase from *E. coli* by interaction with pyridoxal phosphate.

Bacterial RNA degradosome complex

Escherichia coli rRNA, tRNA and mRNA processing and degradation involves a macromolecular complex, the RNA degradosome. The major constituents of the degradosome include RNAse E, PNPase, and a DEAD box helicase and enolase (Rauhut and Klug 1999). Recently, the genes encoding the degradosome components, as well as those involved in the synthesis or interaction with ribonucleotides and sugar compounds, were found to be among the most conserved gene sequences in the three primary domains, suggesting an ancestral origin (Delaye et al. 2005).

Even though PNPase is absent in Archaea, RNase PH is traceable to the last universal common ancestor (LUCA) (Anantharaman et al. 2002). RNAse PH is the other phosphate-dependent exoribonuclease that has been implicated in the processing of tRNA precursors.

A polyphosphate kinase, the enzyme which catalyses the reversible polymerisation of ATP γ-phosphate to polyphosphate and ADP, has also been identified as a component of the *E. coli* RNA degradosome (Blum et al. 1997). Exopolyphosphatase is another enzyme involved in polyphosphate metabolism, releasing Pi from the ends of Poly P (Brown and Kornberg 2004).

Enolase is also a component of the degradosome, but its function in this complex remains unknown. It is a key enzyme in the metabolism of sugars, catalysing the dehydration of 2-phosphoglycerate to phosphoenolpyruvate and the reverse reaction in gluconeogenesis, through a beta-elimination mechanism. A remarkable point is that PNPase coevolved with enolase and glycolysis enzymes in Firmicutes (Danchin 2009).

Enolase is also a nucleic acid binding protein (al-Giery and Brewer 1992) with an all-alpha-helical domain that is present in PNPase (Kuhnel and Luisi 2001). Structural studies of enolase have showed the presence of two magnesium ions, found to be involved in the phosphodiester hydrolysis of double-stranded RNA catalysed by Ribonuclease III (Sun et al. 2005). Nucleic acid polymerases also seem to carry out their catalytic functions through a two-metal ion mechanism that could be conserved in other polymerases (Steitz 1998). It will be interesting to see whether enolase is capable of catalysing the synthesis or degradation of RNA.

Stable bound polymer-phosphorylase complexes

As mentioned earlier, both template-dependent and non-template-dependent processes can lead to the synthesis of biopolymers. Both types of polymerisation reactions seem to require an oligomer (primer) that acts as the initiation point for chain elongation. A number of polymers, including certain viral DNA and RNA genomes, polysaccharides and long-chain fatty acids, are initiated on an oligomer covalently linked to a priming protein.

Aon and Curtino (1984) provided the first evidence for a protein covalently bound to glycogen. Further characterisation studies showed that the glycogenin protein autocatalyses the initiation steps in the synthesis of glycogen by self-glucosylation. The reaction produces a malto-oligosaccharide chain of about 7–11 glucose residues attached to the enzyme that serves as a primer for the glycogen synthase (Lomako et al. 2004). Glycogenin has not been found in bacteria, where the bacterial glycogen is synthesised by glycogen synthase as primer protein (Ugalde et al. 2003).

In the case of PNPase, binding studies during polymerisation and degradation showed the formation of a strong enzyme-polynucleotide complex where the polymer remains attached to the outer surface of the enzyme by multiple subsites (Godefroy-Colburn and Grunberg-Manago 1972).

Rossmann-fold and oligonucleotide/oligosaccharide binding fold

The basic pathways of glycogen metabolism involve the following glycosyltransferases: glycogen-synthetase, glycogenin and glycogen phosphorylase. These enzymes contain at least one nucleotide-binding domain Rossmann-like fold ($\beta\alpha\beta$) which is common to the nucleotide-binding domains of most glycosyltransferases (Gibbons et al. 2002; Buschiazzo et al. 2004). Two Rossmann-folds ($\beta\alpha\beta\beta\alpha\beta$) are present in the structure of the most prevalent RNA-binding proteins (Nagai et al. 1990).

PNPase contains four distinct domains: two core RPH domains (RNase PH), one alpha helix, and two RNA-binding domains, S1 and KH. The S1 domain of PNPase is folded into a five-strand antiparallel β-barrel (Symmons et al. 2000) with all the determinants of the oligonucleotide/oligosaccharide binding (OB-) fold (Bycroft et al. 1997). The OB-fold is a five-stranded β-sheet that forms a β-barrel, and is present in different proteins binding DNA, RNA, oligonucleotides and oligosaccharides (Murzin 1993).

Curiously, PNPase has the GGREGLVD sequence within the catalytic centre and RNA binding site located in the S1 domain. This octapeptide is highly conserved among eubacterial, eukaryotic and archaebacterial DNA-dependent RNA polymerases, suggesting a remote origin, but its function remains unknown (Lazcano et al. 1992).

Polyribonucleotides, polysaccharides and polyphosphates as chemical reservoirs

Polysaccharides are synthesised by polymerisation reactions catalysed by polysaccharide synthases that use ribonucleoside-diphosphate sugar units as substrate. The occurrence of sugar residues combined with ribonucleoside-diphosphate as glycosyl donor is an invariant rule of polysaccharide polymerisation reactions. On the other hand, glycogen phosphorylase is capable of catalysing in vivo breakdown and in vitro synthesis of glycogen.

Polyphosphate kinase catalyses the reversible conversion of the terminal (γ) phosphate of ATP to inorganic polyphosphate and ADP; and a second enzyme, exopolyphosphatase hydrolyses the terminal residues of polyphosphate releasing Pi.

The polymerisation reactions of RNA catalysed by phosphate-dependent exoribonucleases (PNPase and RNase PH) require ribonucleoside-diphosphate as substrates. In the

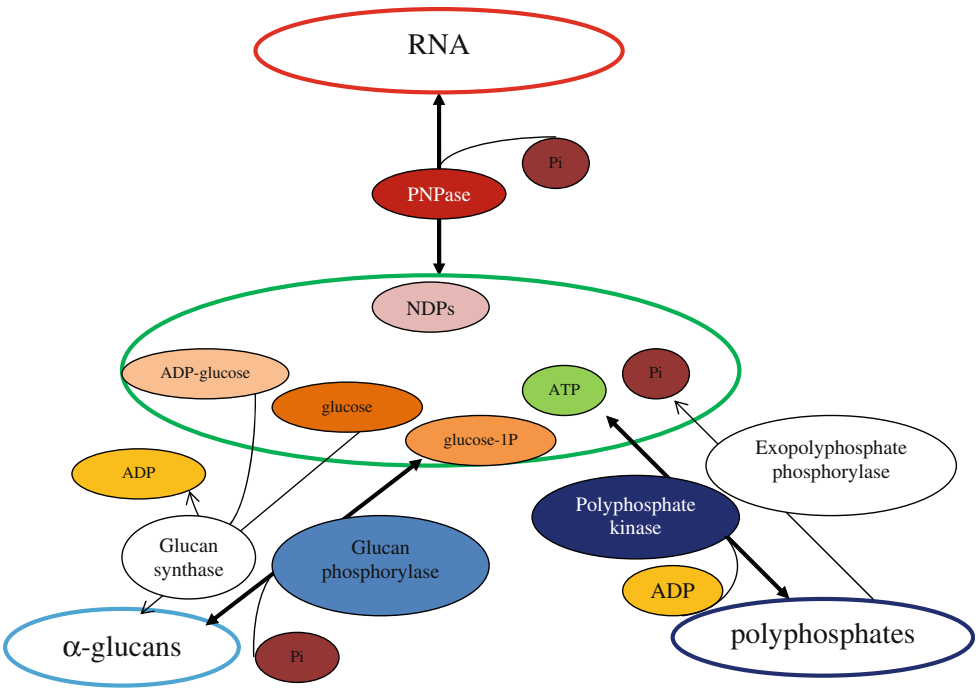

Fig. 1 Non-template-driven polymers: metabolic connections. Glucan, polyribonucleotide and polyphosphate polymers connected by reversible polimerisation reactions catalysed by glucan phosphorylase, PNPase (polynucleotide phosphorylase) and polyphosphate kinase, respectively. The scheme also includes the enzymes glucan synthase and exopolyphosphatase. The reactive units are: ADP-glucose, glucose, ATP, NDP (ribonucleoside diphosphate) and Pi (inorganic phosphate)

reverse reaction, phosphorolytic RNA degradation catalysed by exoribonucleases also releases nucleoside diphosphates.

Modern glucans, RNA and polyphosphates are synthesized by reversible non-template-dependent polymerisation reactions connected in metabolic pathways (Fig. 1). So, it is possible to envisage that ancestral polyribonucleotide, polysaccharide and polyphosphate polymers could have been originated in intertwined pre-biotic systems by similar, reversible and interdependent chemical reactions sharing building blocks, available for reuse.

RNA polymers would have served as a repository of the nucleoside diphosphate cofactors required in the synthesis of polysaccharides. Reciprocally, polysaccharides would have supported sugar monomers for the synthesis of RNA-like polymers, and polyphosphate would have provided nucleosides triphosphates necessary for the synthesis of RNA and polysaccharides, and phosphate for their phosphorolytic degradation (Fig. 2).

The reversibility and robustness of these reactions would have been a selective advantage for the development of proto-metabolism that allowed the emergence of informative polymers through error tolerance or error correction polymerisation mechanisms.

α-Glucans adopt double-helical structures

In amylopectin and glycogen, both highly branched α-glucans (α-1,4-glucosidic bonds) the glucose units of the chains tend to undergo helical coiling. The helix contains six residues per turn (Metzler 2001). In amylose, a linear α-glucan, a tightly coiled double-helical form has been proposed (Kainuma and French 1972). Each chain would also contain six glucose units per turn and the two chains could be arranged either parallel or antiparallel, including the formation of hairpin folds.

Contrary to β-glucans with β-sheet structures, polymers of sugar monomers connected by α-glycosidic bonds can naturally adopt the configuration of an α-helix. The ability

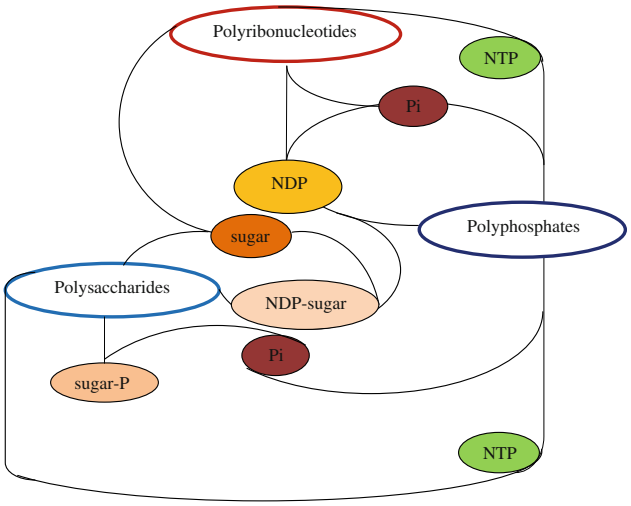

Fig. 2 Non-template-driven polymers: chemical connections. Synthesis and degradation of polysaccharide, polyribonucleotide and polyphosphate polymers would have been intertwined in pre-biotic systems by similar and interdependent chemical reactions sharing reactive compounds: sugars, NDP (ribonucleoside diphosphate), NDP-sugars, NTP (nucleoside triphosphate) and Pi (inorganic phosphate)

to form chains and pairings would have provided a basic self-organisation, essential for polymerisation reactions contributing to the emergence of a template activity, as suggested for the template-dependent DNA duplication process by Watson and Crick (1953b).

RNA ribose-phosphate backbone is similar to oligosaccharides

Recently, Kitamura et al. (2005) proposed a model for the origin of RNA based on a physico-chemical theory of polymerisation in a liquid–gas transition. This model predicts that the RNA polymerisation of nucleotides by a 2′–5′ or 3′–5′ phosphodiester linkage, to form the ribose-phosphate backbone, is similar to oligosaccharides and polysaccharides. This prediction suggests a physico-chemical similarity between phosphodiester in RNA and the glucosidic chain in polysaccharides.

Glycogen has also been found to contain phosphodiester groups incorporated by UDPglucose-glycogen glucose 1-phosphotransferase (Lomako et al. 1993), showing that this type of bond is also present in α-glucans.

Nucleoside formation: nucleobase-sugar connection

Several alternative hypotheses explain the prebiotic origin of the glycosidic bond (N, O-acetal) that connects the nucleobase to ribose. Some consider that nucleobases were formed on pre-existing sugars. For example (Sanchez and Orgel 1970) demonstrated the synthesis of cytidine monophosphate from 5′-phopshorylated ribose.

On the other hand, sugars synthesised on nucleobases have been obtained with formamide in the presence of montmorillonite. The reaction produced a whole range of acylonucleosides, nucleobases accompanied by *N*-formylpurine containing a masked glucosidic bond (Saladino et al. 2004). The glycosidic bond can react with other sugar moieties, as the clay mineral has been also shown to catalyse aldol condensations of formaldehyde into sugars by the so-called formose reaction. It has been postulated that, under prebiotic conditions, nucleoside diphosphates could have been synthesised along with standard nucleotides (Schwartz and Orgel 1985).

Initiators and primers in the synthesis of RNA

DNA and RNA template-dependent polymerases require a primer complementary to the template that is the point of elongation of the new chain. In contrast, the reactions catalysed by PNPase appear to use the primer only as an initiator of the polymerisation reaction. It has been observed that the primer is not necessarily incorporated in the polymer (Chou et al. 1975) and acts as an initiator that allows the production of the same polymer as in glycogen auto-catalysis (Cori and Cori 1939).

First replicator(s) from non-template-driven polymerisations

For most theories on the origin of life based on a metabolic-first scenario, it is plausible to imagine a starting point from non-template-dependent processes. More difficult to envisage is the replication-first paradigm, where the appearance of replicators as self-copying and template molecules seem more restrained. One way to circumvent such hypothetical restrictions is to consider that the first replicator(s) would have originated from non-template-dependent processes, to later become replicative. It was recently contended, from phylogenomic analysis and ontological data, that metabolic functions preceded replicative functions, and that if an RNA world existed it was metabolic, not replicative (Kim and Caetano-Anollés 2010).

In non-enzymatic RNA polymerisation experiments, polynucleotide templates containing cytidine have been found to facilitate the synthesis of oligonucleotides and direct the synthesis of its complement (Orgel 2004). However, the efficiency of monomer incorporation decreases when the cytosine content decreases. These features were considered a restriction for poly C to act as a template to direct repeated rounds of replication.

In the case of RNA polymerisation catalysed by PNPase, poly C is a universal primer. It primes its own synthesis and that of all polyribonucleotides (Mii and Ochoa 1957). Beljanski (1996) reported that the specificity of PNPase was reduced and that the enzyme catalysed the polymerisation of deoxyribonucleotides under in vitro conditions and in the presence of $FeCl_3$, and attempted to analyse his observations from an evolutionary perspective.

Polymer phosphorylases as primitive polymerases used in precellular models

Following the pioneer observations by Ochoa, who demonstrated in the late 1950s that, under in vitro conditions, PNPase could catalyse the template-free synthesis of RNA molecules, PNPase, as a polysaccharide phosphorylase, was seen as a model of a primitive polymerase. For example, Oparin and co-workers studied the enzymatic polymerisation of glucose-1P catalysed by potato phosphorylase (Oparin et al. 1962) and polymerisation of ADP

catalysed by PNPase (Oparin et al. 1963) with his so-called coacervates as a model of pre-biotic cells.

Oparin's work with polymer phosphorylases has been revisited on several occasions, by different research groups, with micelles and self-reproducing vesicules as the most relevant models for precellular systems (Oberholzer and Luisi 2002). These experimental approaches pointed to a primal role of both phosphorylases in the emergence of organised systems.

Discussion

Some hypotheses contemplate the relevance of non-template-dependent processes in the appearance of life on Earth. A carbohydrate world as the origin of life has been suggested, considering the reactivity, energy, and the natural propensity of carbohydrates to polymerise (Weber 2005). Other hypotheses, based on the thermodynamic incompatible miscibility of macromolecules, propose that polysaccharides might have been the first biopolymers to act as compatibilisers and concentrators of other polymers under physico-chemical constraints (Tolstoguzov 2004). Stern and Jedrzejas (2008) also hypothesised a glyco-world with catalytic carbohydrates such as clodextrins, in which carbohydrate polymerisation was facilitated by high-energy polyphosphates to become self-replicating and scaffold for the assembly of other macromolecules.

Recently, studies on the metabolic origins of modern molecular functions have revealed that cellular biopolymer metabolic process (Gene Ontology database: 0034960, defined as the chemical reactions and pathways involving biopolymers carried out by individual cells), or chemosynthesis, was the most ancient biological process, preceding the formation of biopolymers and RNA biosynthesis. These results suggest a metabolism-first scenario, with the production of informational polymers in the absence of biosynthetic pathways by pure chemical reactions (Kim and Caetano-Anollés 2010).

Within the genetic-first view of life, Joyce (2002) has suggested an RNA-later hypothesis in which simpler polymers such as threose nucleic acid, peptide nucleic acid, glycerol-derived nucleic acid analogue and pyranoyl-RNA nucleotide base emerged by pre-biotic chemistry and evolved to RNA.

Christian de Duve (1995) has designated, under the name of proto-metabolism, a stable network of pre-biotic chemical reactions capable of generating and sustaining the RNA world for the development of RNA replication, protein synthesis and translation.

Other hypotheses on the origin of life postulate that genes and metabolism emerged together by co-evolution (Copley et al. 2007). Geochemical processes established pre-biotic reaction networks gradually enriched by acquisition of catalytic compounds and chemical pathways. From the very beginning, this proto-metabolism with intertwined metabolic and replicative process became more effective and complex, allowing the emergence of living systems.

Similarly, as mentioned above, synthesis of ancestral RNA and polysaccharide polymers might have shared reactive intermediates and synthetic pathways, and might have emerged from common polymerisation reactions as a consequence of a high degree of connectivity between carbohydrate and RNA pre-biotic chemistries. More importantly, the assumption of an ancestral non-template-dependent polymerisation process reconciles the gene-first and metabolism-first hypotheses.

On the other hand, the PNPase enzyme seems to be a functional palimpsest of polysaccharide phosphorylases, RNA polymerases, RNases and nucleotidyltransferases. It shares phosphorolytic activity and non-template-dependent polymerisation with polysaccharide phosphorylases. It is related to nucleic acid polymerases in the formation of pyrophosphate bonds and the synthesis of polynucleotides, and to RNAses in RNA degradation. PNPase also belongs to the superfamily of nucleotidyl transferases where the inorganic orthophosphate acts as acceptor. All these functional similarities are probably indicative of an ancient origin for PNPase and intertwined chemical reactions from the beginning.

Although sequence and structural analyses provide no evidence for a common origin shared by PNPase and glycogen phosphorylase, it is tempting to assume that an ancient polymerisation activity for polynucleotide and polysaccharide formation may have preceded the emergence of polymerases. This ancestral polymerisation function could have become specialised to yield the different non-template-dependent and template-dependent activities of contemporary polysaccharides and nucleic acids.

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