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De Novo Synthesis of DNA-Like Molecules by Polynucleotide Phosphorylase In Vitro

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Abstract. In the presence of Mg^{2+} ions, polynucleotide phosphorylase (PNPase, EC 2.7.7.8) is known to synthesize RNA-like polymers using ribonucleoside-5′-diphosphate (NDP) substrates but to be unable to utilize deoxyribonucleoside substrates. Our experiments show that when $MgCl_2$ is replaced by $FeCl_3$, PNPase becomes able to synthesize deoxyheteropolymers using deoxyribonucleoside-5′-diphosphates (dNDPs). The deoxyheteropolymer formed from the four dNDPs is degraded by pancreatic DNase, but not by RNase, and is readily used as a template by DNA-dependent DNA polymerase. Synthesis of this DNA-like polymer is accomplished de novo without the help of any primer or preexisting template. What is more, dA/dG and dC/dT ratios of polymers synthesized by different bacterial PNPases closely match ratios found in DNA of the bacterial species the enzyme came from.

Key words: Polynucleotide phosphorylase — De novo deoxypolymer synthesis — DNA — Deoxyribonucleoside-5′-diphosphates — Ferric ions

Introduction

Among the many enigmas which obscure the emergence of the living cell, DNA synthesis is perhaps the most challenging. Explanations, some involving purely abiotic means, have been offered for the formation of protein and RNA (Fox 1974; Eigen et al. 1981; Orgel 1986; Brack and Raulin 1991; Brack 1994; Barbieri 1981; Biebricher 1993; Ferris 1994). But the appearance of the DNA molecule, which reliably replicates identically to itself so as to be the repository of the inborn characters of the cell, still remains largely unaccounted for. This paper attempts to shed some light on this evolutionary step.

DNA synthesis as we know it today is a purely replicative process involving the concerted activity of a number of enzymes and requiring the presence of a primer and a preexisting template. The most elusive aspect of DNA emergence is the nature of its original template. The favored explanation is that DNA was copied from already-existing RNA with the help of the then-newly-created, but-today-ubiquitous, reverse transcriptase. Yet could there be another solution?

On several occasions in the course of my research, I investigated, together with my former collaborators, the properties of the enzyme polynucleotide phosphorylase (PNPase) (Beljanski and Beljanski 1968; Beljanski et al. 1970). Its discoverer in 1956, Severo Ochoa (Ochoa 1956, 1957), demonstrated that, in vitro and in the presence of $MgCl_2$, PNPase assembled monoribonucleotides into homopolymers or heteropolymers, and to this end it would use exclusively the diphosphonucleotides (NDP: namely, ADP, GDP, CDP, UDP). Heteropolymers formed by PNPase from these four ribonucleotides could not be chemically distinguished from natural RNAs; yet the role of the enzyme in RNA synthesis in vivo was never demonstrated. PNPase, which soon ceased to arouse researchers' interest, was, however, detected in various microorganisms (Ochoa 1956, 1957; Beers 1956,

Abbreviations: PNPase, polynucleotide phosphorylase; dNDP, deoxyribonucleoside-5′-diphosphates; NDP, ribonucleoside-5′-diphosphates; DNA, deoxyribonucleic acid; RNA, ribonucleic acid

1957), in plants (Brummond et al. 1957; Vardanis and Hochster 1961), and in mammalian tissues (Hilmoe and Heppel 1957).

Iron, like magnesium, plays a pivotal role in many fundamental biological processes, and notably in DNA synthesis: it is required, for instance, by most ribonucleotide reductases, which convert ribonucleotides into deoxyribonucleotide building blocks (Reichard 1993), as well as by vertebrate egg reverse transcriptase (Beljanski et al. 1988). Replacement of ferrous by ferric (Fe^{3+}) ions, when living organisms had given off sufficient oxygen into the atmosphere, is considered a major turning point in evolution (Lewin 1984).

We decided to investigate whether the presence of $FeCl_3$, instead of $MgCl_2$, would modify PNPase syntheses and choice of substrates. In this paper, we present results of experiments using PNPases from different bacterial species and demonstrate that, in the presence of $FeCl_3$, these enzymes become able to utilize deoxyribonucleoside-5′-diphosphates (dNDP) and to synthesize de novo, without the help of any primer, a DNA-like molecule; moreover, in the synthesized polymers, the purine/pyrimidine ratio closely matches that of genomic DNA of the bacterium each enzyme came from.

Materials and Methods

Unlabeled substrates: 2′-deoxyribonucleoside-5′-diphosphates, ribonucleoside-5′-diphosphates: Sigma, St. Louis, MO, USA, and Boehringer Mannheim S.A., Meylan, France. Labeled substrates: ^{14}C-ADP (195 mCi/mM), ^{14}C-GDP (168 mCi/mM), ^{14}C-CDP (206 mCi/mM), ^{14}C-UDP (182 mCi/mM): Schwartz Bioresearch Inc., Orangeburg, NY, USA. ^{14}C-dADP (46 mCi/mM), ^{14}C-dGDP (40 mCi/mM), ^{14}C-dCDP (45.9 mCi/mM), ^{14}C-dTDP (42 mCi/mM); ^{3}H-dADP (16.6 Ci/mM), ^{3}H-dGDP (12.4 Ci/mM): Amersham France S.A., Les Ulis, France.

Polynucleotide phosphorylases: from *Micrococcus lysodeikticus*, lyophilized, sp. act. 49 units/mg protein (280/260 nm = 1.1): PL Biochemical, Inc., Pabst Laboratories, Milwaukee, WI, USA; from *Escherichia coli*, lyophilized, sp. act. 30 units/mg protein (280/260 nm = 1.30): Sigma Chemical Co., St. Louis, MO, USA; from *Alcaligenes faecalis*, purified in our laboratory, sp. act. 40 units/mg protein (280/260 nm = 1.05) (Beljanski et al. 1970). Ratio of UV absorptions at 280 and 260 nm serves to detect the eventual presence of RNA or DNA in proteins (Warburg and Christian 1942).

Pancreatic RNase A, RNase-free DNase: Worthington Co., USA. Alkaline phosphatase, polynucleotide kinase: Sigma France. $FeCl_3$, crystallized: Prolabo, Paris. Fe(III)OH: Aldrich Chemical Co, Milwaukee WI, USA. $Fe_2(SO_4)_3$, $NH_4Fe(SO_4)_2$: Sigma France. Egg albumin (soluble): Difco Laboratories, Detroit, MI, USA. All other chemicals, grade A: Prolabo, Paris, or Merck France. Whatman GF/C glass filter: WR Balsom, England. Sephadex G-100: Pharmacia France. DNA molecular weight marker V, digexigenin labeled: Boehringer Mannheim, Biochemical catalog 1995. (γ^{32}P)-ATP (3000 Ci/mM): Amersham, England.

1. *Incorporation of ^{14}C-labeled deoxyribonucleotides from dNDP.* Incubation medium (0.25 ml) contained: substrate(s), nM concentrations indicated in Tables 1 and 2; Tris-HCl buffer (pH 7.65): 25 mM; $FeCl_3$: 0.05 mM; PNPase: depending on its origin and the experiment, from 0.45 to 0.73 units/assay or more, as indicated. Incubation: 10 min at 37°C. Reaction was stopped by addition of an equal volume of cold trichloroacetic acid (5% TCA) and 50 μg egg albumin. Precipitate was filtered on a glass GF/C filter, washed with TCA solution, then with 95% ethanol, and dried. Radioactivity was measured using 3 ml of scintillation fluid and a scintillation spectrometer. Results are expressed as cpm of TCA-precipitable material.
2. *Synthesis of larger amounts of deoxyheteropolymer to allow characterization.* Incubation medium (0.5 ml per tube) contained: dNDP, 1.25 mM each; crystalline $FeCl_3$: 1.5 mM; *M. lysodeikticus* PNPase: 14.7 units; Tris-HCl buffer (pH 7.65): 150 mM; toluene: 0.02 ml. Care must be taken to precisely adjust pH to indicated value. Number of tubes was increased as needed for preparation of the required amount of deoxypolymer. Tubes were covered with Parafilm and incubated at 37°C for 1–6 h. Following incubation, an excess volume of 95% alcohol and 0.1 ml of 1 mM KCl solution were added to incubation medium. Mixture was kept at −20°C for 2 h, then cold-temperature centrifuged, and precipitate was dialyzed. Deoxypolymer was filtered on a Sephadex G-100 column (35 × 1 cm) equilibrated with Tris-HCl (0.1 mM, pH 7.4); 2-ml fractions were collected and 260-nm UV absorbance was determined. Synthesized polymer was then further purified using chloroform treatment followed by dialysis against Tris-HCl buffer (1 mM, pH 7.4, containing 0.1 M NaCl). In additional experiments, after incubation at 37°C followed by alcohol precipitation, preeipitate was dissolved in buffer and treated twice with chloroform. The aqueous phase was extensively dialyzed against Tris-HCl buffer.

Purified deoxyheteropolymer 260/280 UV absorbance ratio was then measured and the diphenylamine colorimetric reaction (a specific stain for deoxyribose) was performed (Burton 1965). Molecular size was estimated by polyacrylamide gel electrophoresis (as described in caption to Fig. 1).

Results

$FeCl_3$-Induced Modification of NDP Utilization by PNPase

Under the usual experimental conditions for ribopolymer in vitro synthesis, i.e., using mM concentrations of the four required NDPs in the presence of Mg^{2+} ions (added as mM $MgCl_2$), the enzyme synthesizes an acid-precipitable, RNA-like molecule (Ochoa 1956, 1957; Beers 1956). However, if substrate amounts are decreased to nM values, PNPase becomes inactive (Table 1), in spite of the presence of Mg^{2+} ions. If $MgCl_2$ is now replaced by $FeCl_3$, the enzyme becomes able to utilize nM NDP concentrations (Table 1). In this instance, $FeCl_3$ concentrations as low as 0.05 mM prove active.

Novel, $FeCl_3$-Induced Utilization of dNDP by PNPase

The inability of PNPase to utilize dNDP as substrates in the presence of Mg^{2+} ions is well documented (Ochoa 1956). This inability persists even when a DNA template is added to the incubation medium.

Yet when Mg^{2+} ions are replaced by Fe^{3+} from $FeCl_3$, PNPase becomes able to utilize dNDP, and in as low as nM amounts. Other sources of ferric ions, such as $Fe_2(SO_4)_3$ and $NH_4Fe(SO_4)_2$, also induce enzyme activ-

Table 1. Formation in vitro of homopolymers from ^{14}C-ribonucleoside-5′-diphosphates and ^{14}C-deoxyribonucleoside-5′-diphosphates by *E. coli* PNPase in the presence of $MgCl_2$ and $FeCl_3$[a]

Substrate	cpm	
	$MgCl_2$ (0.05 mM/assay)	$FeCl_3$ (0.05 mM/assay)
^{14}C-ADP	196 ± 46	2,266 ± 262
^{14}C-GDP	164 ± 74	2,809 ± 472
^{14}C-CDP	233 ± 58	2,378 ± 374
^{14}C-UDP	246 ± 64	2,552 ± 302
^{14}C-d-ADP	129	2,051 ± 57
^{14}C-d-GDP	63	1,798 ± 183
^{14}C-d-CDP	125	1,866 ± 92
^{14}C-d-UDP	113	1,946 ± 109

[a] Incubation medium (0.25 ml) contained: Tris-HCl buffer, pH 7.65: 25 mM; ^{14}C-NDP: 30,000 cpm each; ^{14}C-dNDP: 30,000 cpm each; $MgCl_2$: 0.05 mM; $FeCl_3$: 0.05 mM; *E. coli* PNPase: 0.25 units. Incubation in a water bath for 10 min at 37°C. TCA-precipitable material was filtered on a Millipore GF/C filter, washed with 5% TCA solution and then with alcohol, and dried, and radioactivity was measured. Results, expressed in cpm, are mean values of three independent experiments

ity. Due to its extremely low water solubility, Fe(III)OH is not an adequate Fe^{3+} source. Enzyme activity may be easily evaluated using labeled substrates (Table 1). Single deoxyribonucleoside-5′-diphosphates are utilized to form homopolymers; when the four dNDPs are provided, a deoxyheteropolymer containing the two purine and two pyrimidine bases is repeatedly formed.

An appreciable amount of this deoxyheteropolymer could already be obtained following a 1-h incubation, medium surface being protected by toluene from airborne contamination. Polymer was purified (see Materials and Methods) and characterized. Several independent experiments showed it had a 260/280-nm UV absorption ratio of 1.76–2.2, demonstrating its nucleic acid nature; diphenylamine reaction, which specifically detects deoxyribose, was positive. Polyacrylamide gel electrophoresis indicated a molecular size of at least 600 bp (Fig. 1).

Synthesis increased when $FeCl_3$ was increased from 0.6 to 1.8 mM. Other biologically active ions such as Cu^{2+}, Zn^{2+}, Co^{2+}, or Mn^{2+} (assayed as chlorides) were unable to trigger polymerization of deoxyribonucleotides from dNDP. We checked that when dNDPs were incubated with either $FeCl_3$ or protein (egg albumin) or both together, but in the absence of PNPase, no polymer was formed.

Measurement of deoxyheteropolymer 260-nm UV absorbance after KOH denaturation yielded hyperchromicity values ranging, among various samples, from 24% to 54%, that of native DNA being 55% (see Discussion). Deoxyheteropolymer was easily degraded by pancreatic DNase, but not by RNase A. Like DNA, and in contrast to RNA, it was not degraded by KOH under the usual experimental conditions. However, we observed that addition of Fe^{3+} ions (from $FeCl_3$) unexpectedly brought about complete KOH degradation not only of the deoxyheteropolymer, but also of native DNA as well. As may be seen from Fig. 2, the DNA-like deoxyheteropolymer (here synthesized by *M. lysodeikticus* PNPase) was readily used as a template by DNA-dependent DNA polymerase under classic experimental conditions.

Differential Polymerization of the Various Deoxyribonucleotides from dNDP by Three Different PNPases

Polymer synthesis in the presence of $FeCl_3$ was carried out using enzymes from three different bacterial species:

Table 2. Purine and pyrimidine ratios of deoxyribopolymers synthesized in vitro by different PNPases in the presence of $FeCl_3$[a]

^{14}C-labeled substrates	*Micrococcus lysodeikticus* PNPase		*Escherichia coli* PNPase		*Alcaligenes faecalis* PNPase	
	cpm	Ratio	cpm	Ratio	cpm	Ratio
d-ADP	6,716 ± 577		7,847 ± 219		4,816 ± 288	
		dA/dG : 0.47		dA/dG : 0.90		dA/dG : 0.57
d-GDP	13,696 ± 460		8,675 ± 259		8,676 ± 650	
d-CDP	8,940 ± 416		4,898 ± 589		8,949 ± 229	
		dC/dT : 2.29		dC/dT : 1.08		dC/dT : 2.07
d-TDP	3,897 ± 186		4,498 ± 462		4,316 ± 527	
d-ADP + d-GDP	20,726 ± 768		15,446 ± 756		13,410 ± 936	
d-CDP + d-TDP	11,499 ± 656		8,128 ± 563		11,986 ± 612	
dADP + d-GDP + d-CDP + d-TDP	30,478 ± 1,976		23,202 ± 1,747		24,964 ± 1,732	
	In bacterial DNA:	dA/dG : 0.39 dC/dT : 2.52	In bacterial DNA:	dA/dG : 0.95 dC/dT : 1.04	In bacterial DNA:	dA/dG : 0.49 dC/dT : 1.94

[a] Incubation medium (0.25 ml) contained: Tris-HCl buffer, pH 7.65: 25 mM; ^{14}C-dADP, ^{14}C-dGDP, 14C-dCDP, ^{14}C-dTDP: 50,000 cpm each; these substrates were incubated first separately (yielding homopolymers), then two, and finally four together as indicated. $FeCl_3$: 0.06 mM; PNPases from *Micrococcus lysodeikticus, Alcaligenes faecalis,* or *Escherichia coli:* 0.73, 0.60, and 0.45 units, respectively (for further processing, see Materials and Methods). Results, expressed in cpm, are mean values of three independent experiments

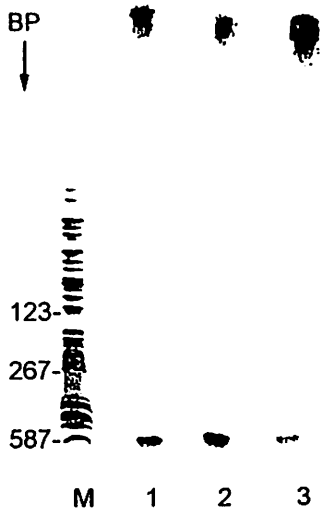

Fig. 1. Deoxyheteropolymer molecular size evaluation. Deoxyheteropolymer was synthesized by *Micrococcus lysodeikticus* PNPase using equimolar amounts of the four dNDPs and purified as described in Materials and Methods. Incubation: 6 h for samples 1 and 2, and 3 h for sample 3 (synthesized in three independent experiments). For ^{32}P labeling, deoxyheteropolymers were treated with polynucleotide phosphatase to remove 5′ terminal phosphate, which was then replaced by 32-phosphate from ($\gamma^{32}P$)-ATP using polynucleotide kinase (EC 3.7.1.78). Polyacrylamide gel electrophoresis (8%) was run on a 8.5 × 11.5 cm gel plate at 150 V for about 2 h, using digexigenin-labeled DNA molecular weight marker V (500 ng), a mixture of 22 fragments obtained by cleavage of plasmid pBR 322 DNA with restriction endonuclease *Hae*III (Sambrook 1989). ^{32}P heteropolymer samples (about 1 μg) show the presence of labeled deoxyheteropolymer containing at least 600 bp. Radioactive bands were visualized by exposing to a Kodak scientific film.

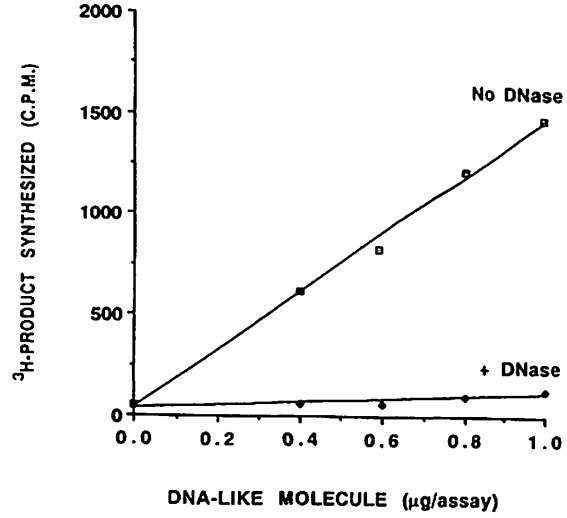

Fig. 2. Template activity of deoxyheteropolymer synthesized in vitro by *Micrococcus lysodeikticus* PNPase in the presence of $FeCl_3$. Incubation medium (0.15 ml) classically contained: Tris-HCl buffer, pH 7.65: 25 mM; $MgCl_2$: 2 mM; the four required dNTPs: 5 nM each; ^{3}H-TTP: 100,000 cpm. *E. coli* DNA-dependent DNA polymerase I: 2.0 units; purified DNA-like deoxyheteropolymer: indicated concentrations; DNase: 40 μg. Following a 40-min incubation at 37°C, TCA-precipitable material was filtered on a GF/C Millipore filter, washed with TCA (5% solution) and then with alcohol, dried, and radioactivity was measured. Results, expressed in cpm, are mean value of three independent experiments.

Micrococcus lysodeikticus, Alcaligenes faecalis, and *Escherichia coli,* and the purine and pyrimidine nucleotide ratios of the deoxyheteropolymer molecules were evaluated.

PNPase from Either *M. lysodeikticus* or *A. faecalis*

1. When, in successive experiments, the enzyme was presented with equimolar, low (nM) amounts of single, labeled dNDP substrates (dADP, dGDP, etc.), the amounts of homopolymers synthesized from each of these substrates differed. The amount of each homopolymer was closely proportional to that of the corresponding mononucleotide present in the DNA of the bacterial species the enzyme came from (Table 2).
2. When from two to four labeled dNDPs were incubated together, heteropolymer counts/min closely corresponded to added counts of corresponding homopolymers (Table 2), with dA/dG and dC/dT ratios in the DNA-like heteropolymer closely matching those found in the DNA of the bacterium each enzyme originated from.

There are stringent conditions to this proportionality: (1) the presence of Fe^{3+} from $FeCl_3$ and (2) low amounts of substrate (ranging, for instance, from 50 to 500 nM for *M. lysodeikticus* PNPase, as indicated in Fig. 3). In contrast, purine and pyrimidine ratios are independent of the amount of enzyme used.

It may be seen from the example for *M. lysodeikticus* given in Fig. 3 that, as the total amount of substrate was increased into the mM range (by addition of unlabeled dNDP to unmodified amounts of labeled dNDP), enzyme selectivity leading to dA/dG similarity in the polymer and bacterial DNA progressively disappeared.

E. coli PNPase

Whereas in *M. lysodeikticus* and *A. faecalis* DNAs, purine and pyrimidine ratios are not equal to 1:1 and selectivity in substrate utilization is required if they are to be the same in the deoxyheteropolymer and bacterial DNA, these ratios are both 1:1 in *E. coli* genomic DNA. Then it might be assumed that less selectivity would be required of the *E. coli* enzyme. Using *E. coli* PNPase, the base ratio of synthesized heteropolymer closely approximated that of bacterial genomic DNA, no matter whether nM or mM substrate concentrations were used. Besides the use of radiolabeling, differential substrate incorporation was checked by measurement of monophosphate release from unlabeled dNDP, under appropriate conditions (data not presented here).

It must be stressed that measurement of the 280/260-

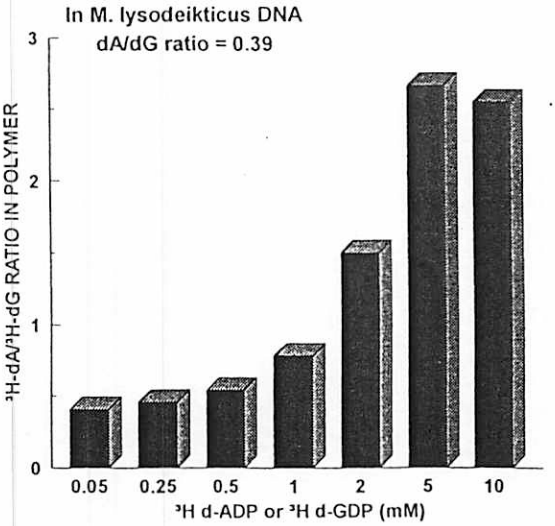

Fig. 3. Nucleotide incorporation from ^{3}H-dADP and ^{3}H-dGDP by PNPase from *Micrococcus lysodeikticus*. For experimental conditions, see Table 2. Total amount of substrate was increased from the nM into the mM range by addition of unlabeled dNDP to unmodified amounts of ^{3}H-labeled dNDP. It may be seen that dA/dG ratio is dependent on substrate amount. Results indicate ratio of labeled A and G nucleotides, which are increasingly diluted as unlabeled nucleotide incorporation increases. Using over 0.5 mM of either dADP or dGDP, incorporation of unlabeled dG increases more than that of dA, so that cpm proportionately decrease for dGDP, and ^{3}H-dA/^{3}H-dG ratio is correspondingly modified. Results are mean values of three independent experiments.

nm UV absorbance ratio (Warburg and Christian 1942) of each enzyme showed there was no nucleic acid contamination and that all our efforts to isolate an eventual DNA template from the enzymes, using phenol and chloroform treatments, failed. The absence of any RNA primer in purified *M. lysodeikticus* PNPase had already been reported (Beers 1958).

Discussion

Ever since its discovery, that is, for 40 years, PNPase has been classified as a ribopolymer-synthesizing enzyme, requiring Mg^{2+} ions and NDP substrates exclusively, and forming molecules chemically undistinguishable from RNA. Yet it was purported to lack RNA-synthesizing activity in vivo. Little or no attention was paid to its presence and role in various bacteria (Beers 1956, 1957, 1958; Ochoa 1956, 1957), plants (Brummond et al. 1957; Vardanis and Hochster 1961), and mammalian cells (Hilmoe and Heppel 1957). Emphasis was laid on RNA polymerase, and PNPase soon became the forgotten enzyme.

However, years ago, we demonstrated (Beljanski and Beljanski 1968; Beljanski et al. 1970) that a PNPase isolated from a showdomycin-resistant strain of *Escherichia coli* could, in the presence of Mg^{2+} ions, synthesize riboheteropolymers having a purine/pyrimidine ratio of 2:1. This was the first intimation we had of the ability of PNPase to achieve differential purine and pyrimidine polymerization.

Recently, we had occasion to uncover another example of this activity, obviously of much greater impact. Over the years, at the hands of numerous researchers, evidence had accumulated pointing to the pivotal role of iron, and notably ferric ions, in fundamental life processes. For instance, ribonucleotide reductase (Follmann 1982; Reichard 1993), which performs on-the-spot conversion of ribonucleotides into deoxyribonucleotides when they are required for DNA synthesis by DNA polymerase, is dependent on Fe^{3+} ions for its activity in plants, animals, and most aerobic bacteria. Ferric ion is a powerful agent: upon its addition in vitro, dormant activity of fish egg reverse transcriptase instantly flares up to high levels (Beljanski et al. 1988). This set us wondering how PNPase would respond to Fe^{3+} ions.

This paper describes the outcome of our in vitro experiments using three different bacterial PNPases and $FeCl_3$ as a source of ferric ions. It may be summed up as follows:

1. The presence of Fe^{3+} [in this instance, from $FeCl_3$; but $Fe_2(SO_4)_3$ and $NH_4Fe(SO_4)_2$ are also active, while ferric hydroxyde, very poorly water-soluble, is inadequate] does modify PNPase properties, in such a way that the enzyme now becomes able to polymerize deoxyribonucleoside-5′-diphosphates (dNDP).
2. A DNA-like polymer is produced using the four dNDPs, without any external help such as an RNA primer, a DNA template, or another enzyme. The DNA-like nature of the synthesized deoxyheteropolymer can be demonstrated by its DNase degradation and its utilization as a substrate by DNA-dependent DNA polymerase can be shown. It must be emphasized that there was no detectable nucleic acid contamination in the enzymes we used. In addition, following KOH denaturation, measurement of deoxyheteropolymer 260-nm UV absorbance showed that samples exhibited hyperchromicity, which is the sign of H-bond breakage, ranging from 24% to 54%, i.e., practically reaching that of native DNA, 55%. As synthesized deoxyheteropolymer is single-stranded, H-bonds must have formed wherever polymer chain contains complementary sequences inducing more or less extensive molecular folding, a frequently occurring process; hyperchromicity variation between samples would then depend on the extent of folding. Ready use of the DNA-like polymer as a template by DNA-dependent DNA polymerase, a primer requiring enzyme, could be explained, for instance, by the presence of a complementary hairpin which could serve as a priming sequence, similarly to what occurs in parvoviruses. Folding of the DNA-like polymer, locally mimicking double strand formation, should take on a

new importance in the search for DNA precursor molecules.

3. Purine and pyrimidine ratios of the DNA-like molecule are affected by substrate concentration: Using nM concentrations of dNDP, PNPases from *M. lysodeikticus* and *A. faecalis* form a deoxyheteropolymer with purine and pyrimidine ratios extremely close to those of the genome of the bacterial species the enzyme came from. This selectivity is independent of the amount of enzyme. With increasing substrate concentrations, selectivity progressively disappears. In contrast, purine and pyrimidine ratios in *E. coli* genomic DNA are both equal to 1:1, and they remain unchanged using *E. coli* PNPase, no matter whether low or high substrate concentrations are used. This decreased need for selectivity might relate to the fact that *E. coli* is reported to be less ancient than *M. lysodeikticus* or *A. faecalis* (Woese 1967).
4. When Fe^{3+} is replaced by Mg^{2+}, PNPase becomes totally unable to use deoxy substrates.
5. Yet the same PNPase that utilized dNDP in the presence of Fe^{3+} can also form RNA-like polymers, provided iron is replaced by magnesium and NDPs are available, high concentrations of both active ion and substrate being this time required (Ochoa 1956; Beers 1956, 1958). It may be noted that, in present-day cells, NDP are from 100- to 1,000-fold more abundant than dNDP and dNTP.

Thus, PNPase can, by itself, de novo, form DNA-like molecules, discriminate between the four dNDPs, and switch to RNA-like production when metal ions (and substrates) are modified.

Certainly many questions arise, notably concerning the enzyme's ability to assemble purine and pyrimidine monomers in a particular ratio (having, so to speak, an inbuilt template function) and to be activated by two different metal ions, and concerning also the precise role of iron and magnesium, all these properties being dependent on PNPase detailed molecular structure, about which too little is presently known. Some of our experiments, as yet preliminary, indicate that iron binds to the deoxynucleotides which are taken up for DNA-like polymer formation.

Various observations plead in favor of the early origin of PNPase, such as its versatility, or its ability to assemble a polymer no matter how many of the four dNDPs are available, contrasting with highly specialized DNA-dependent DNA polymerase and reverse transcriptase, which remain inactive if even just one of the four dNTP substrates they require is missing. In this regard, we must note that DNA polymerase cannot use dNDP, only dNTP (Kornberg et al. 1956; Bessman et al. 1958). So we may wonder: did PNPase play a part in the evolution of DNA?

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