Biochimica et Biophysica Acta, 1000 (1989) 53–56
Elsevier

The early history of DNA polymerase: a commentary by

Arthur Kornberg

Department of Biochemistry, Stanford University School of Medicine, Stanford, CA (U.S.A.)

on 'Enzymic synthesis of deoxyribonucleic acid'
by A. Kornberg, I.R. Lehman, M.J. Bessman and E.S. Simms
Biochim. Biophys. Acta 21 (1956) 197–198

The brief publication [1] in Biochimica et Biophysica Acta (BBA) entitled "Enzymic Synthesis of Deoxyribonucleic Acid", submitted May 2, 1956, was the first such report of DNA synthesis, aside from an abstract which appeared in Federation Proceedings in March of that year [2]. In the BBA paper, we (see photograph, p. 55) showed that the incorporation of [^{14}C]thymidine into an acid-soluble, DNase-sensitive, alkali-stable product depended on partially purified enzyme fractions, ATP and DNA which was converted to an 'active primer'. We expressed the brash hope of assembling 'genetically specific DNA'.

Two years later, we submitted two papers to the Journal of Biological Chemistry describing the 2000-fold purification of DNA polymerase and the enzymatic synthesis of the four deoxyribonucleoside triphosphates which the enzyme assembled into long chains. The papers were titled: 'The Enzymatic Synthesis of DNA. I. Preparation of Substrates and Partial Purification of an Enzyme from *Escherichia coli*. II. General Properties of the Reaction.' The papers were not accepted because of the term 'DNA' in the title. The consensus among the many referees after a protracted review was that the papers would be acceptable if we used the term "polydeoxyribonucleotide" to identify the synthetic product; the use of 'DNA' could be justified only with a demonstration of genetic activity. To us this seemed unreasonable, because few if any DNA papers in the Journal had been required to fulfill this criterion. Fortunately, with accession of John Edsall to the editorship, the papers were accepted with DNA in the title [3,4].

Primer or template

Because the historic papers by Watson and Crick in 1953 [5] proposed that the strands of a DNA double helix could serve as templates for replication, it is commonly assumed that, in my initial search for DNA synthesis in cell-free extracts 2 years later, the DNA I included in the reaction mixture was there to serve that purpose. Not so. I added DNA expecting that it would serve as a primer for growth of a DNA chain because I was influenced by the work of Carl and Gerty Cori on the growth of a carbohydrate chain by glycogen phosphorylase. In the BBA paper [1] we refer to the added DNA as a source of 'active primer'. I never thought that I would discover a phenomenon utterly unprecedented in biochemistry: an absolute dependence of an enzyme for instruction by its substrate serving as a template.

I had added DNA for another reason. Nuclease action in the extracts was rampant, and I wanted a pool of DNA to surround and protect at least some of the newly incorporated thymidine. Only later did we learn with elation that the added DNA fulfilled two other essential roles. It served as a template and also as a source of the missing nucleotides. The DNA was cleaved by DNases in the extract to nucleotides. These were converted by ATP and five kinases in the extract to the di- and triphosphates of the A, G, C and T deoxyribonucleosides, which were then still unknown. It was not until 1970 that we realized that DNA polymerase cannot start a chain and requires a primer to do so.

Today, when we recognize that the first DNA polymerase, found in *E. coli*, is prototypical for all DNA polymerases and is widely used in the analysis and engineering of genes and chromosomes, it may be worth recalling crises of doubt and confusion, as well as triumphs, that surrounded this enzyme in its early history.

Synthesis without an apparent template

Having become convinced of the absolute need of the polymerase for a template, we were shocked to discover the massive synthesis of DNA without any added template. After a lag period of hours, the purified enzyme made DNA-like polymers of simple composi-

0006-3002/89/$03.50

tion [6,7]: the alternating copolymers poly(dA · dT) and poly(dG · dC) and the homopolymer pairs poly(dA) · poly(dT) and poly(dG) · poly(dC). These polymers, once made, proved to be superior templates and have been widely used in DNA chemistry and biology. Subsequent studies showed that generation of the polymers de novo could be ascribed to the reiterative replication of short sequences in the immeasurably small amounts of DNA that contaminate a polymerase preparation [8,9].

Nuclease in the polymerase

Knowing that DNA polymerase synthesized a chain in the 5′ to 3′ direction, it made no sense to me then that the presumably pure enzyme degraded the very 3′ end of the chain it would normally be extending. In the absence of the deoxynucleoside triphosphates needed for synthesis, nucleotide units were cleaved slowly and serially from the 3′ end of a DNA chain. Eventually, we learned that the enzyme removes frayed or mismatched units at the end of a primer, thereby permitting fresh units to be added to the growing chain end only when it is correctly matched to the template chain. We could infer that if the synthesizing enzyme were to make a rare mistake during elongation of a chain, such as inserting a C opposite an A (estimated to happen once in ten thousand times), it would remove the mismatched C before proceeding with extension of the chain. This astonishing proofreading ability of the enzyme [10], coupled with its fine discrimination in the initial choice of correct building blocks during synthesis, reduces errors in the overall process of replication to one in ten million.

Having finally made sense of why an activity that degrades DNA is part of the very enzyme that makes it, we were unprepared for the observation that nuclease activity in DNA polymerase persists even when digestion from the 3′-end is blocked by an analog or phosphate [11,12]. Upon examining this activity, we found that a separate domain in the enzyme operates at a nick in a duplex to remove nucleotides from the 5′ end of a chain. Then it became clear that polymerase could use this other nuclease function to remove foreign structures in DNA, such as the thymine dimer lesion of ultraviolet irradiation [13], and as we recognized some years later, could perform an essential step in replication by removing the RNA that initiates the start of a DNA chain.

Synthesis of genetically active DNA

For more than 10 years after the BBA paper [1], I had to search for excuses at the end of every seminar to explain why our DNA product had no biologic activity. If the template had been copied accurately, why were we unsuccessful in all our attempts to multiply the transforming factor activity of DNA from *Pneumococcus, Hemophilus*, and *Bacillus* species? Finally, with the discovery of DNA ligase in 1967, a crucial test could be made. We replicated the single-stranded circle of phage ϕX174 with DNA polymerase and then sealed the complementary product with ligase. The circular product strand was isolated and then replicated to produce a circular copy of the original viral strand, which could now be assayed for infectivity in *E. coli* [14]. We found the completely synthetic viral strand to be as infectious as that of the phage DNA we started with [15]!

After so many years of trying, we had gotten DNA polymerase to assemble a 5000-nucleotide DNA chain with the identical form, composition, and genetic activity of DNA from a natural virus. All the enzyme needed was the four A, G, T and C deoxynucleoside triphosphates. At that moment, it seemed there were no major impediments to the synthesis of DNA, genes and chromosomes. The way was open to create novel DNA and genes by manipulating the building blocks and their templates.

DNA polymerase cannot start a chain

Despite the excitement over the synthesis of a chain of infectious viral DNA, I had felt a certain uneasiness. One of the inferences drawn from the replication of a single-stranded, circular template was that DNA polymerase could start a new chain. Yet we were never able to find direct proof of this. Moreover, we had observed that replication of the circular template was far more efficient if a small amount of boiled *E. coli* extract was present. Although it seemed unlikely that a random fragment of DNA in the extract would match the viral DNA template accurately enough to serve as a primer, this proved to be the case. DNA polymerase removed the unmatched regions of the partially annealed fragment by proofreading at the 3′ end; with generous editing at the 5′ end, no trace of the fragment remained in the synthetic product.

We were left with the question of how a DNA chain is started, how a single-stranded, circular viral DNA is converted to the duplex form upon entering the cell, and how nascent chains are initiated in the replication of virtually all chromosomes. Indeed, Okazaki had shown earlier [16] that chains are started not just once, at the beginning of the chromosome, but repeatedly in staccato fashion during the progress of replication.

DNA polymerase under indictment

DNA polymerase was called a 'red herring' and charged by Nature New Biology in a series of editorials with masquerading as a replication enzyme [17]. The replicative role of DNA polymerase was questioned because of the Cairns mutant of *E. coli* [18], which appeared to lack the enzyme and yet grew and multipled at a normal rate. In addition to the apparent dispensability of DNA polymerase for cell multiplication and its more estimable qualifications for repair of

The Microbiology Department of Washington University in St. Louis 1956–1957. Among those present are the four authors of the BBA paper [1].
Left to right: Front row: J. McLeary, K. Horibata, M. Walsh, J. Hurwitz, *A. Kornberg*, E. Battley, D. Hogness;
Middle row: *I.R. Lehman*, M. Bartsch, A.D. Kaiser, S. Johnson, M. Cohn, P. Berg, *E. Simms*, J. Ofengand, G. Bugg;
Back row: H. Wiesmeyer, O. Ward, E. Holmes, D. Daniels, H. Morales, L. McKeown, *M. Bessman*, E. Stonehill, V. Johnson.

DNA, the enzyme could not start a chain and genes were being discovered (designated *dnaA, dnaB, dnaC,* etc.) which strongly implicated many other proteins as essential for a replication process far more complex than we had imagined.

With the rising skepticism about the importance of DNA polymerase, there was serious questioning about the validity of the basic mechanism, the building blocks and the assays of DNA synthesis. At this juncture, a new polymerase was found in *E. coli*. Named DNA polymerase II (pol II) [19], it was clearly different from the 'classic' DNA polymerase (pol I) and from still another DNA polymerase III (pol III) [20] discovered in the course of purifying pol II. Pol III, in a far more elaborate form, was to gain recognition as the central enzyme of DNA replication in *E. coli*.

All three polymerases, although differing significantly in structure, proved to be virtually identical in their mechanisms of DNA synthesis, proofreading and use of the same building blocks. The maligned pol I became the standard for all DNA polymerases in plants, animals and viruses, as well as in *E. coli*. The gloomy views of Nature New Biology soon disappeared, as did the magazine itself.

Primosomes, helicases and replisomes

In 1972, after a 10-year drought of discoveries of new enzymes, they came in a torrent. Probing the initiation and elongation of DNA of the single-stranded circles of the small coliphages (M13, G4, ϕX174), illuminated the extraordinarily complex assembly of proteins (primosomes) that initiate chains, the multisubunit DNA polymerase III holoenzyme that elongates them and the helicases that open duplexes to expose the template for replication [21,22]. Similarly, the intensitive studies of phages T4 [23,24] and T7 [25] and of animal viruses [26,27] have revealed the fascinating variations on conserved basic themes of replication. Still unclear and of great importance is the cellular spatial organization and temporal regulation of these entities. We imagine a superassembly of the replication proteins which can effect essentially concurrent synthesis of both strands at a replication fork. We call it a 'replisome', but it has yet to be taken alive.

References

1 Kornberg, A., Lehman, I.R., Bessman, M. and Simms, E. (1956) Biochim. Biophys. Acta 21, 197–198.
2 Kornberg, A., Lehman, I.R. and Simms, E. (1956) Fed. Proc. 15, 291.
3 Lehman, I.R., Bessman, M.J., Simms, E.S. and Kornberg, A. (1958) J. Biol. Chem. 233, 163–170.
4 Bessman, M.J., Lehman, I.R., Simms, E.S. and Kornberg, A. (1958) J. Biol. Chem. 233, 171–177.
5 Watson, J.D. and Crick, F.H.C. (1953) Nature 171, 737–738; 964–967.
6 Schachman, H.K., Adler, J., Radding, C.M., Lehman, I.R. and Kornberg, A. (1960) J. Biol. Chem. 235, 3242–3249.
7 Radding, C.M., Josse, J. and Kornberg, A. (1962) J. Biol. Chem. 237, 2869–2876.
8 Radding, C.M. and Kornberg, A. (1962) J. Biol. Chem. 237, 2882.
9 Kornberg, A., Bertsch, L., Jackson, J.F. and Khorana, H.G. (1964) Proc. Natl. Acad. Sci. USA 51, 315–323.
10 Brutlag, D. and Kornberg, A. (1972) J. Biol. Chem. 247, 241–248.
11 Klett, R.P., Cerami, A. and Reich, E. (1968) Proc. Natl. Acad. Sci. USA 60, 943–950.
12 Deutscher, M.P. and Kornberg, A. (1969) J. Biol. Chem. 244, 3029–3037.
13 Kelly, R.B., Atkinson, M.R., Huberman, J.A. and Kornberg, A. (1969) Nature 224, 495–501.
14 Goulian, M. and Kornberg, A. (1967) Proc. Natl. Acad. Sci. USA 58, 1723–1730.
15 Goulian, M., Kornberg, A. and Sinsheimer, R.L. (1967) Proc. Natl. Acad. Sci. USA 58, 2321–2328.
16 Sugimoto, K., Okazaki, T. and Okazaki, R. (1968) Proc. Natl. Acad. Sci. USA 60, 1356–1362.
17 Editorials (1971) Nature New Biol. 229, 65–66; 230, 258; 233, 97–98.
18 De Lucia, P. and Cairns, J. (1969) Nature 224, 1164–1166.
19 Kornberg, T. and Gefter, M.L. (1970) Biochem. Biophys. Res. Commun. 40, 1348–1355.
20 Kornberg, T. and Gefter, M.L. (1971) Proc. Natl. Acad. Sci. USA 68, 761–764.
21 Kornberg, A. (1980) DNA Replication, p. 724, W.H. Freeman & Co., San Francisco.
22 Kornberg, A. (1988) J. Biol. Chem. 263, 1–4.
23 Nossal, N.G. (1983) Annu. Rev. Biochem. 52, 581–615.
24 Selick, H.E., Barry, J., Cha, T.-A., Munn, M., Nakanishi, M., Wong, M.L. and Alberts, B.M. (1987) Replication and Recombination: UCLA Symposia on Molecular and Cellular Biology, New Series, pp. 183–214, Alan R. Liss, New York.
25 Tabor, S., Huber, H.E. and Richardson, C.C. (1987) J. Biol. Chem. 262, 16212–16223.
26 Li, J.J. and Kelly, T.J. (1985) Mol. Cell. Biol. 5, 1238–1246.
27 Rosenfeld, P.J., O'Neill, E.A., Wedes, R.J. and Kelly, T.J. (1987) Mol. Cell. Biol. 7, 875–886.

Correspondence: A. Kornberg, Department of Biochemistry, Beckman Center, Stanford University, Stanford, CA 94305-5307, U.S.A.

Enzymic synthesis of deoxyribonucleic acid

We have reported[1] the conversion of ^{14}C-thymidine via a sequence of discrete enzymic steps to a product with the properties of DNA*.

$$\text{Thymidine} \xrightarrow{\text{ATP}} \text{T5P} \xrightarrow{\text{ATP}} \text{TTP} \xrightarrow{\text{ATP}} \text{"DNA"} \qquad (1)$$

The thymidine product is acid-insoluble, destroyed by DNAase, alkali-stable and resistant to RNAase. We have now extended these studies to include adenine, guanine and cytosine deoxynucleotides, and with partially purified enzymes from *E. coli* we have studied further the nature of the polymerization reaction.

^{32}P-labeled deoxynucleotides were prepared by enzymic digestion of DNA obtained from *E. coli* grown in a ^{32}P-containing medium; the nucleotides were then phosphorylated by a partially purified enzyme. The principal product of T5P phosphorylation was separated as a single component in an ion-exchange chromatogram and identified as TTP. The ratios of thymidine:acid-labile P:total P were 1.00:2.03:3.08. Enzymic formation of the di- and triphosphates of deoxyadenosine and the pyrimidine deoxyribonucleosides has been observed[2] and the presence of pyrimidine deoxyribonucleoside polyphosphates in thymus extracts has been reported[3].

Polymerization of TTP requires ATP, a heat-stable DNA fragment(s), provisionally regarded as a primer, and two enzyme fractions (called S and P; previously[1] called A and B, respectively) each of which has thus far been purified more than 100-fold (Table I). Preliminary studies suggest that TDP can replace TTP and has the same requirements for incorporation into DNA; a decision as to the more immediate precursor requires further purification of the system.

"Primer" for the *crude* enzyme fraction was obtained (1) by the action of crystalline pancreatic DNAase on *E. coli* DNA or (2) on thymus DNA, or (3) by an *E. coli* enzyme fraction (SP) acting on DNA contained in it. However, "primer" for the *purified* enzyme fraction was obtained only with method (3); the action of pancreatic DNAase on either *E. coli* or thymus DNA did not yield "primer". These findings imply the existence of an activity in the crude enzyme fraction responsible for the formation of active "primer". The chemical properties of the unpurified "primer" resemble those of a partial digest of DNA.

Utilization of the polyphosphates (presumably triphosphates) of adenine, guanine and cytosine deoxynucleosides for DNA synthesis occurs at rates approximately equal to those for TTP in crude enzyme fractions, but at appreciably slower rates with the enzyme purified for TTP polymerization (Table II). These changes in ratio suggest the presence of different enzymes for each of the deoxyribonucleoside triphosphates. Mixtures of these triphosphates, each tested at concentrations near enzyme saturation, gave additive or superadditive rates, further suggesting different enzymes for each of the substrates and a facilitation of polymerization by such mixtures.

Studies are in progress to define the mechanism of the polymerization reaction and the

* Abbreviations used are: DNA, deoxyribonucleic acid; ATP, adenosine triphosphate; T5P, thymidine-5′-phosphate; TDP, thymidine diphosphate; TTP, thymidine triphosphate; DNAase, deoxyribonuclease; RNAase, ribonuclease.

linkages and sequences in the DNA-like product formed. Further investigations with phage-infected *E. coli*[1] and studies with biologically active DNA may begin to clarify the question of how genetically specific DNA is assembled.

TABLE I

REQUIREMENTS OF THE PURIFIED SYSTEM

Extracts of *E. coli B* prepared by sonic disintegration were treated with streptomycin to yield a precipitate (fraction SP) and a supernatant fluid (fraction SS). Ammonium sulfate, gel and acid fractionation procedures applied to fractions SP and SS yielded fractions P and S, respectively. *E. coli* DNA was prepared by heating fraction SP (optical density at 260 mμ = 15) at 70° for 10 minutes. To produce "primer", 0.1 ml of *E. coli* DNA was combined with 40 γ of fraction SP; after 1 hour at 37° in the presence of $5 \cdot 10^{-3} M$ $MgCl_2$, the mixture was heated for 10 minutes at 80°. The complete system contained (in 0.3 ml) 0.014 μmole of TTP ($1.5 \cdot 10^6$ c.p.m./μmole), 0.1 μmole of ATP, 0.10 ml of "primer", 10 γ of fraction S, 1 γ of fraction P, 1 μmole of $MgCl_2$, and 20 μmoles of glycine buffer, pH 9.2. After incubation for 30 minutes at 37°, 0.05 ml of crude *E. coli* extract ("carrier") and 0.3 ml of 7% perchloric acid were added. The precipitate was washed, plated and its radioactivity measured.

	mμmoles DNA-P/hour
Complete system	1.48
No ATP	0.20
No "primer"	0.11
No enzyme fraction S	0.07
No enzyme fraction P	0.04

TABLE II

CONVERSION OF FOUR DEOXYNUCLEOSIDE TRIPHOSPHATES

The incubation mixtures and assays were as described in Table I except that (1) the concentrations of deoxynucleoside triphosphates were $1.5 \cdot 10^{-5} M$, and (2) the crude enzymes were 60 γ of fraction SP and 240 γ of fraction SS.

Triphosphates	*Tested with crude enzymes*	*Tested with purified enzymes (for TTP)*
	mμmoles DNA-P/hour	
Thymidine (T)	0.8	5.48
Deoxyguanosine (G)	0.6	0.98
Deoxycytidine (C)	0.8	1.44
Deoxyadenosine (A)	0.6	1.28
T + G	2.2	14.6
T + G + C	4.4	19.6
T + G + C + A	6.4	22.0*
T + G + C + A (no "primer")	2.0	0.28

* 65% conversion of substrate.

Fellowship support of I. R. LEHMAN by the American Cancer Society, of M. J. BESSMAN by the Public Health Service, and grants to A. KORNBERG by the Public Health Service and the National Science Foundation are gratefully acknowledged.

Department of Microbiology, Washington University School of Medicine, St. Louis, Mo. (U.S.A.)

ARTHUR KORNBERG
I. R. LEHMAN
MAURICE J. BESSMAN
E. S. SIMMS

[1] A. KORNBERG, I. R. LEHMAN AND E. S. SIMMS, *Federation Proc.*, 15 (1956) 291.
[2] H. Z. SABLE, P. B. WILBER, A. E. COHEN AND M. R. KANE, *Biochim. Biophys. Acta*, 13 (1954) 156. L. I. HECHT, V. R. POTTER AND E. HERBERT, *ibid.*, 15 (1954) 134.
[3] R. L. POTTER AND S. SCHLESINGER, *J. Am. Chem. Soc.*, 77 (1955) 6714.

Received May 2nd, 1956