Purification and Characterization of Two Forms of DNA Polymerase α from HeLa Cell Nuclei†

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ABSTRACT: Isolated nuclei contained two active forms of DNA polymerase α (form I and form II). Form II was extracted from nuclei by KCl at concentrations lower than 0.18 M. Above 0.18 M selective extraction of form I was observed. The purified two active forms differed in chromatographic and electrophoretic behaviors, in their salt requirement for optimal activity, and in preference of template-primers, although both forms exhibited properties characteristic of DNA polymerase α such as sensitivity of *N*-ethylmaleimide, 1-β-D-arabinofuranosylcytosine triphosphate, and aphidicolin. Marked difference between the two forms was preference of template-primer that form I was more active with poly(dT)·$(rA)_{10}$ than poly(dA)·$(dT)_{12}$ whereas form II exhibited higher activity with poly(dA)·$(dT)_{12}$ than poly(dT)·$(rA)_{10}$. Possible roles of two forms of DNA polymerase α in the processes of DNA replication will be discussed.

Although many enzymes and other protein factors implicated in DNA replication of mammalian cells have been isolated and characterized, their precise roles in the processes of DNA replication are still unclear. One of the promising approaches to this problem has been the development of reconstituted nuclear systems (Seki & Mueller, 1976; Brun & Weissbach, 1978; Tanuma et al., 1980). We have shown (Tanuma et al., 1980; Enomoto et al., 1983) that by mild salt treatment of isolated nuclei at appropriate concentrations, factors necessary for some steps of DNA replication can be selectively solubilized from isolated nuclei, leaving salt-treated nuclei capable of carrying out other steps of DNA replication. Attempts to purify replication factors and to reconstitute replication processes with these factors are necessary to elucidate the molecular mechanism of mammalian DNA replication.

As for one of the essential factors in DNA replication, DNA polymerase, DNA polymerase α is suggested to be involved in DNA replication among the three DNA polymerases, α, β, and γ, found in mammalian cells (Weissbach, 1977). In various eukaryotic cells, the existence of multiple forms of DNA polymerase α, which differ in their chromatographic behavior, has been demonstrated (Yoshida et al., 1974; Hachmann & Lezius, 1975; Craig & Keir, 1975; Matsukage et al., 1976; PedraliNoy & Weissbach, 1977; Nishioka et al., 1977; Bieri-Bonniot & Schuerch, 1978; Hesselewood et al., 1978; Brakel & Blumenthal, 1978; Chen et al., 1979; Lamothe et al., 1981). We have also reported that HeLa cells or their isolated nuclei contain two active forms of DNA polymerase α (Ono et al., 1978, 1979; Tanuma et al., 1980). Although such heterogeneity is probably intrinsic property of DNA polymerase α, the biological significance of the heterogeneity is unknown.

In this report, in an attempt to assess the relevance of the existence of two forms of DNA polymerase α to the processes of DNA replication, we have analyzed the composition of DNA polymerases in nuclear extracts and have partially purified and characterized two forms of DNA polymerase α. To separate and purify the two forms efficiently, we have developed a new method, "nuclear-cellulose column". We will discuss the functional difference of the two forms of DNA polymerase α.

Materials and Methods

Materials. Activated calf thymus DNA was prepared according to the procedure of Aposhian & Kornberg (1962). Poly(rA)·$(dT)_{12}$, poly(dA)·$(dT)_{12}$, and poly(dT)·$(rA)_{10}$ were made by annealing the corresponding polymers with oligomers in a molar nucleotide ratio of 5 to 1.

Buffers. Buffer A contained 10 mM Tris-HCl,[1] pH 7.8, 3 mM $MgCl_2$, 1 mM Na_2EDTA, 2 mM 2-mercaptoethanol, and 0.1 mM phenylmethanesulfonyl fluoride (PMSF). Buffer B contained 40 mM Tris-HCl, pH 7.8, 2 mM 2-mercaptoethanol, 0.1 mM PMSF, and 10% ethylene glycol. Buffer C contained 40 mM Tris-HCl, pH 8.1, 2 mM 2-mercaptoethanol, 0.1 mM PMSF, and 30% ethylene glycol. Buffer D contained 40 mM Tris-HCl, pH 7.8 2 mM 2-mercaptoethanol 0.1 mM PMSF, 50% ethylene glycol, and 30 mM KCl.

Cell Culture, Synchronization, and Preparation of Nuclei. HeLa cells were cultured and synchronized as described previously (Tanuma et al., 1979). Nuclei were isolated from HeLa cells 3 h after the release from hydroxyurea block as described in the preceding paper (Enomoto et al., 1983).

Preparation of Nuclear Extracts. All operations were carried out at 0–4 °C. Isolated nuclei were suspended at concentration of 8×10^7/mL in buffer A containing various concentrations of KCl. After keeping at 0 °C for 60 min, the suspensions were centrifuged at 105000*g* for 60 min at 4 °C.

Assay for DNA Polymerase Activity. (A) DNA Polymerase α Assay. Thirty microliters of reaction mixture contained 50 mM Tris-HCl, pH 7.5, 2.5 mM $MgCl_2$, 2 mM 2-mercaptoethanol, 200 μg/mL bovine serum albumin, 500 μg/mL activated calf thymus DNA, 100 μM each of dATP, dCTP, and dGTP, and 20 μM [^{3}H]dTTP (0.67 Ci/mmol). Incubation was carried out for 30 min at 37 °C. Each reaction mixture was spotted on a 1-in. square of DEAE paper (Whatman DE 81), and the paper was washed with 5% Na_2HPO_4 3 times and then with water, ethanol, and acetone once each.

(B) DNA Polymerase β Assay. Thirty microliters of assay mixture contained 50 mM Tris-HCl, pH 8.5, 10 mM $MgCl_2$,

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[1] Abbreviations: araCTP, 1-β-D-arabinofuranosylcytosine triphosphate; ddTTP, 2′,3′-dideoxythymidine triphosphate; DEAE, diethylaminoethyl; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.

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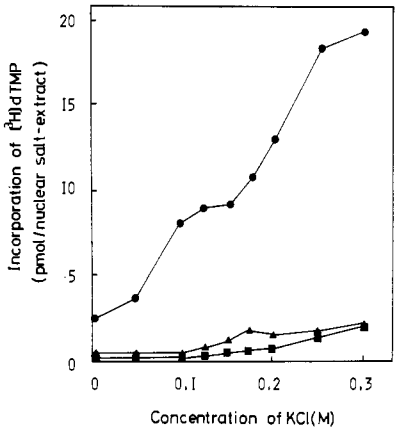

FIGURE 1: Extraction of DNA polymerases from isolated nuclei. Nuclear extracts were prepared as described under Materials and Methods. Two microliters of the nuclear extracts was assayed in a total 30 μL of the reaction mixture for α (●), β (■), and γ (▲) DNA polymerase assay.

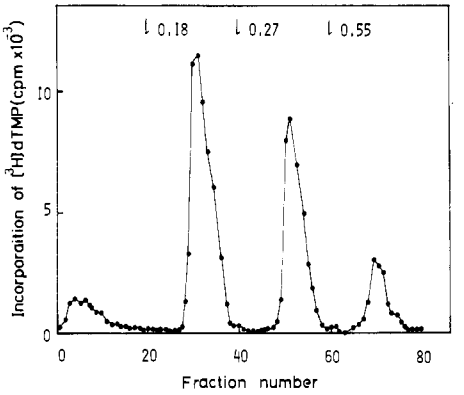

FIGURE 2: Separation of two forms of DNA polymerase α activity by "nuclear-cellulose" column chromatography. For details see text. The column was washed with buffer A–2% ethylene glycol and eluted stepwise with 0.18, 0.27, and 0.55 M KCl in buffer A–20% ethylene glycol successively. Each fraction was assayed for DNA polymerase α.

50 mM KCl, 10 mM *N*-ethylmaleimide, 200 μg/mL bovine serum albumin, 500 μg/mL activated DNA, 100 μM each of dATP, dCTP, and dGTP, and 20 μM [^{3}H]dTTP (0.67 Ci/mmol). The reaction mixture containing enzyme preparation was kept at 0 °C for 30 min and then incubated at 37 °C for 30 min.

(*C*) *DNA Polymerase γ Assay.* Thirty microliters of assay mixtures contained 50 mM Tris-HCl, pH 8.0, 50 mM potassium phosphate, pH 8.0, 50 mM KCl, 0.5 mM $MnCl_2$, 2 mM 2-mercaptoethanol, 200 μg/mL bovine serum albumin, 50 μM [^{3}H]dTTP (0.5 Ci/mmol), and 50 μg/mL poly(rA)·$(dT)_{12}$. Incubation was carried out at 37 °C for 30 min. One unit of polymerase activity is defined as the amount catalyzing the incorporation of 1 nmol of labeled deoxyribonucleotide into acid-insoluble materials in 60 min at 37 °C.

Nondenaturing Polyacrylamide Gel Electrophoresis. Samples were dialyzed against buffer D. Gels were prepared as described by Davis (1964). The upper gel contained 125 mM Tris-HCl, pH 6.8, 2 mM 2-mercaptoethanol, and 20% ethylene glycol. The lower gel buffer contained 375 mM Tris-HCl, pH 8.8, 2 mM 2-mercaptoethanol, and 20% ethylene glycol. Electrophoresis was carried out at a current of 2 mA/gel at 4 °C. After electrophoresis, the gels were stained with 0.3% Coomassie brilliant blue (R-250). The parallel unstained gels were cut into 1.1-mm slices, and DNA polymerase was extracted from the gel slices by incubating each slice for 24 h at 4 °C in 50 μL of buffer D. Recovery of the enzyme activity was 50–80%.

Other Methods. Protein was quantitated according to the method of Lowry et al. (1951) or by the estimation of Coomassie brilliant blue staining intensity in polyacrylamide gels with bovine serum albumin as a standard.

Results

Identification of DNA Polymerase in Nuclear Salt Extract. In the preceding paper (Enomoto et al., 1983), we have shown that by the treatment of isolated nuclei with buffer solutions containing various concentrations of KCl, DNA synthesizing activity in the isolated nuclei was drastically decreased at KCl concentrations above 0.15 M, and the activity was reduced to less than 50% and 10% of original level at 0.2 and 0.3 M KCl, respectively. So we characterized nuclear extracts extracted with KCl at concentrations in the range from 0 to 0.3 M in regard to DNA polymerases.

As shown in Figure 1, DNA polymerase activity in the nuclear extracts measured under the conditions favoring DNA polymerase α increased with KCl concentration and reached a plateau at around 0.25 M. The extraction curve exhibited a biphasic profile with the transition point at around 0.18 M. DNA polymerase activity in the nuclear extracts was inhibited more than 95% by 10 mM *N*-ethylmaleimide. Little activity of DNA polymerase β and γ was detected in these nuclear extracts.

Purification of Two Active Forms of DNA Polymerase α. Step 1: Nuclear-Cellulose Column Chromatography. Isolated nuclei were packed between two layers of cellulose (Whatman CF 11), and the nuclear-cellulose column was washed with buffer A containing 2% ethylene glycol. The concentrations of KCl for the elution of DNA polymerase were set at 0.18, 0.27, and 0.55 M based on the elution profile of two forms of DNA polymerase α from single-stranded DNA cellulose column (Ono et al., 1979; Tanuma et al., 1980) and extraction curve from isolated nuclei (Figure 1). As shown in Figure 2, this stepwise elution resulted in separation of DNA polymerase α into two fractions, one eluted at 0.18 M and the other at 0.27 M. DNA polymerase β was eluted in the 0.55 M fraction. DNA polymerase α form I and form II hereafter designate two activities of α with high- and low-binding affinity to chromatin, respectively. The two forms of DNA polymerase α eluted from nuclear-cellulose column were pooled separately and diluted with buffer B to reduce the KCl concentration to 0.05 M (fraction I).

Step 2: Blue Sepharose Column Chromatography. Fraction I of form I and form II were applied separately on Blue Sepharose columns which were equilibrated with 0.05 M KCl–buffer B. The columns were washed with the same buffer and eluted with a linear gradient of KCl from 0.05 to 0.5 M in buffer B. Form I and form II were eluted from the columns as a single peak (Figure 3). DNA polymerase γ activity contaminated in fraction I was eluted at 0.28 M KCl in both columns. Peak fractions of α activity which contained no γ activity were combined and dialyzed against 0.05 M KCl–buffer C (fraction II).

Step 3: Single-Stranded DNA–Cellulose Column Chromatography. The dialysate (fraction II) was applied on a single-stranded DNA–cellulose column which was previously equilibrated with 0.05 M KCl–buffer C and then eluted with a linear gradient of KCl from 0.05 to 0.5 M in buffer C. As shown in Figure 4, form I (Figure 4A) was eluted at 0.22 M KCl and form II (Figure 4B) at 0.18 M KCl. The peak fractions were pooled and dialyzed against buffer D (fraction III).

Table I: Purification of Nuclear DNA Polymerase α, Form I and Form II[a]

step	total protein (mg)		total act. (unit)		sp act. (units/mg)		yield (%)	
	I	II	I	II	I	II	I	II
nuclear cellulose[b]	19.8	28.6	580	662	29	23	100	100
Blue Sepharose	1.1	2.4	304	373	276	155	52	56
DNA-cellulose	0.1	0.2	92	120	920	600	16	18
gel electrophoresis[c]	0.004	0.01	19	37	4750	3700		

[a] DNA polymerase activity was assayed as described under Materials and Methods with activated DNA as a template primer. One unit of activity is defined as the amount catalyzing the incorporation of 1 nmol of labeled dTMP into acid-insoluble materials in 1 h at 37 °C. [b] Nuclear cellulose was made with 2×10^9 nuclei. [c] A portion of DNA-cellulose fraction was subjected to nondenaturing gel electrophoresis. The amount of protein and the activity were determined by densitometry of stained gel and by elution from the slices of parallel unstained gel, respectively.

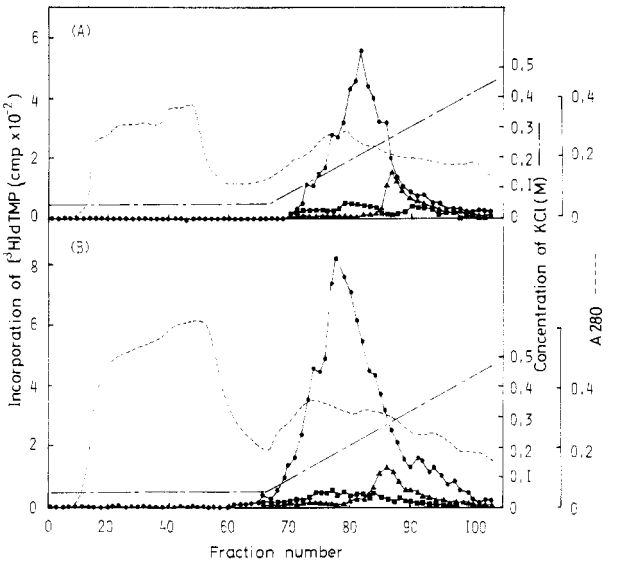

FIGURE 3: Blue Sepharose column chromatography. Fraction I of form I (A) and form II (B) were chromatographed as described in the text. An aliquot of each fraction was assayed for DNA polymerase α (●), β (■), and γ (▲).

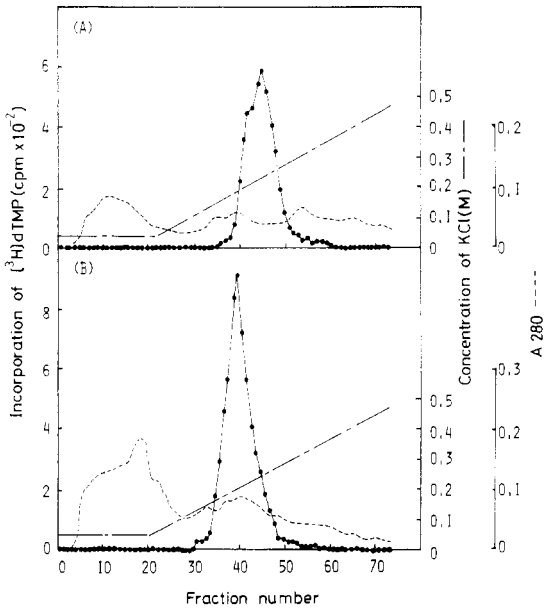

FIGURE 4: Single-stranded DNA–cellulose column chromatography. Fraction II of form I (A) and form II (B) were chromatographed as described in the text. An aliquot of the fraction was assayed for DNA polymerase α.

Step 4: *Nondenaturing Polyacrylamide Gel Electrophoresis.* A portion of fraction III was subjected to nondenaturing polyacrylamide gel electrophoresis. Polymerase activity was eluted from the gel slices (Fraction IV) and assayed with activated DNA as a template-primer. A single major peak of polymerase activity was observed with both forms. Form I (Figure 5A) migrated faster than form II (Figure 5B). Purification of the two forms of DNA polymerase α is summarized in Table I.

Properties of Two Forms of DNA Polymerase α. Eluates from nondenaturing polyacrylamide gels (fraction IV) were used for enzymatic characterization. Specific activity of form I and form II was 4750 and 3700 units/mg of protein, respectively.

Form I and form II had very similar pH optimum, between 7.0 and 7.5. Both forms showed similar divalent cation requirement. The optimal concentrations of Mg^{2+} and Mn^{2+} for both forms were 2.5 and 0.5 mM, respectively. Both activities were inhibited completely by 10 mM EDTA. Neither form showed an absolute requirement for sulfhydryl reducing agents, although both were almost completely inhibited by *N*-ethylmaleimide (Table II). In the presence of 2.5 mM Mg^{2+}, spermidine stimulated form I activity slightly at 1–3 mM, whereas at the same concentrations, it had inhibitory effect on form II activity, and at 5 mM, it reduced the activity by 50%.

The effect of inhibitors on the activities of form I and form II are shown in Table II. Aphidicolin, a specific inhibitor of DNA polymerase α (Ohashi et al., 1978), inhibited both forms.

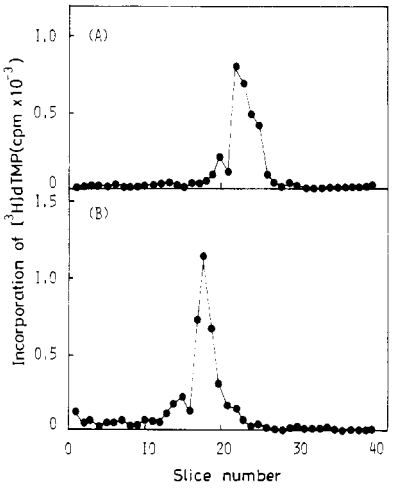

FIGURE 5: Nondenaturing polyacrylamide gel electrophoresis. Fraction III of form I (A) and form II (B) were electrophoresed as described under Materials and Methods. Gels were sliced, DNA polymerase activity was eluted from each slice with buffer D, and the activity was assayed with the DNA polymerase α assay system. The origin of the gel is at the left.

Both forms were very sensitive to *N*-ethylmaleimide in contrast to DNA polymerase β. araCTP inhibited form II more strongly than form I. ddTTP, a potent inhibitor of DNA polymerase β and γ, had little effect at the concentrations tested.

Table II: Effect of Inhibitors on the Activity of DNA Polymerase α, Form I and Form II[a]

inhibitor	relative act. (%) form I	form II
none	100	100
aphidicolin		
10 μg/mL	24.8	35.3
30 μg/mL	10.4	14.0
N-ethylmaleimide		
3 mM	6.1	3.1
10 mM	3.4	2.5
araCTP		
0.3 mM	12.4	3.3
1.0 mM	4.1	1.2
ddTTP		
30 μM	89.2	93.5
100 μM	70.3	69.4

[a] DNA polymerase α activity was assayed as described under Materials and Methods with activated DNA as a template-primer. No addition of inhibitor is defined as 100%. 100% of form I and form II correspond to 7.4 and 14.5 pmol of dTMP incorporation, respectively.

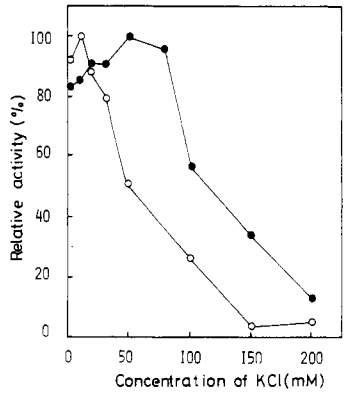

FIGURE 6: Effect of salt on the activities of form I and form II. Assay was performed under the conditions for DNA polymerase α in the presence of indicated concentrations of KCl for 30 min at 37 °C. Values are normalized with respect to each maximal activity as 100%. Form I (●); form II (○).

Double-reciprocal plots of both forms exhibited straight lines giving K_m values for dTTP, 1.8 μM and 2.6 μM for form I and form II, respectively.

Optimal salt concentration differed between the two forms (Figure 6). Form II was inhibited by the addition of KCl higher than 30 mM, and the activity reached 50% of the maximum at 50 mM KCl. Only 5% of the activity remained at 150 mM as compared to the activity at 5 mM KCl. In contrast, form I was stimulated by salt and exhibited maximal activity at KCl concentration around 50–80 mM. At higher salt concentrations, form I was inhibited, and the activity was reduced to less than 50% of the maximum at 150 mM KCl.

Template-primer preferences of the two forms were tested with a variety of natural and synthetic polynucleotides (Table III). Neither form incorporated deoxyribonucleotides in the absence of template-primer. Both forms efficiently used activated DNA. Form II showed a slight preference for native DNA to heat-denatured DNA, whereas form I utilized native and heat-denatured DNA with equal efficiency. The marked difference of the two forms was their abilities to use poly[d-(A-T)], poly(dA)·$(dT)_{12}$, and poly(dT)·$(rA)_{10}$. Form I used poly[d(A-T)] much more efficiently than form II. Form I was most active with poly(dT)·$(rA)_{10}$, whereas form II was most active with poly(dA)·$(dT)_{12}$. Neither form used poly(rA)·$(dT)_{12}$.

Table III: Template-Primer Preference of DNA Polymerase α, Form I and Form II[a]

template-primer	form I [pmol (%)]	form II [pmol (%)]
activated DNA	5.30 (100)	15.0 (100)
native DNA	0.58 (11)	1.50 (10.0)
heat-denatured DNA	0.56 (11)	0.72 (4.8)
poly[d(A-T)]	3.10 (58.5)	2.94 (19.6)
poly(dA)·$(dT)_{12}$	2.90 (54.7)	19.6 (131)
poly(dT)·$(rA)_{10}$	5.94 (112)	9.54 (63.6)
poly(rA)·$(dT)_{12}$[b]	0.22 (4.2)	0.26 (1.7)

[a] Fifteen micrograms of activated, native, and heat-denatured DNA and 1.5 μg of synthetic polymers were used. DNA polymerase activity with activated, native, or heat-denatured DNA was assayed by α assay system as described under Materials and Methods. The activities with synthetic polymers were assayed under the conditions of α assay except that nucleotide triphosphates in the reaction mixture were changed as follows: 100 μM dATP and 50 μM [^{3}H]dTTP for poly[d(A-T)], 50 μM [^{3}H]dTTP for poly(dA)·$(dT)_{12}$, and 50 μM [^{3}H]dATP for poly(dT)·$(rA)_{10}$. [b] Assayed under γ assay condition.

Discussion

We have previously observed that HeLa cells contain two forms of DNA polymerase α distinct in their binding affinity for DNA (Ono et al., 1978, 1979; Tanuma et al., 1980) and that the activity of the one form with higher binding affinity changed with a very close temporal relationship to in vivo DNA synthesis (Ono et al., 1979). We designated these forms as P-I and P-II by the order of elution from the DEAE-cellulose column. When the cells were fractionated into cytoplasm and nuclei under the conditions as described previously (Ono et al., 1978), P-I and P-II were recovered from nuclei and cytoplasm, respectively. The ratio of activity of P-I to P-II was about 0.25. Under the condition used here to isolate nuclei, about 60% of DNA polymerase α activity distributed in cytoplasm and the rest in nuclei. From chromatographic behaviors and enzymological properties, form I corresponded to P-I and form II corresponded to P-II.

"Nuclear-cellulose column" used here separated polymerase α activity into two fractions representing approximately equal amount of activity, and on subsequent chromatographies and electrophoresis, there was no indication of further heterogeneity in each fraction. Even though the nuclear-cellulose column did not provide extensive purification of the two forms, it seems to be useful for preparative method to separate the two forms for further purification.

The purified two forms are categorized into DNA polymerase α because of their sensitivity to aphidicolin (Ohashi et al., 1978), araCTP, and *N*-ethylmaleimide and their template-primer preference. The fact that most purified fractions of the two forms differed in their optimal concentration of KCl, and the abilities to copy various synthetic template-primers, seems to suggest that these differences are derived from the intrinsic properties of the two forms. The possibility that the two forms are generated artificially seems unlikely because coelectrophoresis of both forms in a nondenaturing gel gave two peaks corresponding to form I and II.

Although the biological significance of the existence of two forms of DNA polymerase α is not clear yet, the heterogeneity of DNA polymerase α may correlate with their functions in the processes of DNA replication. The well-known DNA polymerases of *Escherichia coli*, DNA polymerase III holoenzyme, and DNA polymerase I have been suggested to be involved in the synthesis of Okazaki fragments and in the gap filling between these fragments, respectively (Kornberg, 1980). Thus it seems possible that comparable DNA polymerases

might exist in mammalian cells.

DNA polymerase α form I was distinguished from form II by its marked preference for RNA primer. In addition, only one form, form I, was retained in 0.2 M KCl treated nuclei which can synthesize Okazaki fragments whose synthesis was sensitive to aphidicolin (Tanuma et al., 1980). These results strongly suggest that form I is responsible for the synthesis of Okazaki fragments. This possibility was further supported by the results from the reconstitution experiments as described in the preceding paper (Enomoto et al., 1983), showing that the synthesis of Okazaki fragments was restored in 0.3 M KCl treated nuclei by adding back 0.3 M KCl nuclear extract from 0.2 M KCl treated nuclei, which was enriched with form I. However, an attempt to reconstitute the synthesis of Okazaki fragments by adding back the purified DNA polymerase α form I to 0.3 M KCl treated nuclei was not successful. Additional replication factors may be necessary to establish this.

By the reconstitution of nuclear system with 0.2 M KCl treated nuclei and their complementary nuclear extract, the joining step of Okazaki fragments was restored in 0.2 M KCl treated nuclei (in the preceding paper), and this step was shown to be sensitive to aphidicolin (unpublished results). The complementary nuclear extract was enriched with form II. These observations seem to suggest that form II is involved in the gap-filling step between adjecent Okazaki fragments, although more critical experiments are necessary to confirm this.

Registry No. Poly(dT)·$(rA)_{10}$, 27156-07-6; poly(dA)·$(dT)_{12}$, 24939-09-1; araCTP, 13191-15-6; *N*-ethylmaleimide, 128-53-0; aphidicolin, 38966-21-1; DNA polymerase, 9012-90-2.

References

Aposhian, H. V., & Kornberg, A. (1962) *J. Biol. Chem. 237*, 519–525.

Bieri-Bonniot, F., & Schuerch, A. R. (1978) *FEBS Lett. 96*, 192–196.

Brakel, C. L., & Blumenthal, A. B. (1978) *Eur. J. Biochem. 88*, 351–362.

Brun, G., & Weissbach, A. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5931–5935.

Chen, Y.-C., Bohn, E. W., Planck, S. R., & Wilson, S. H. (1979) *J. Biol. Chem. 254*, 11678–11687.

Craig, R. K., & Keir, H. M. (1975) *Biochem. J. 145*, 225–232.

Davis, B. J. (1964) *Ann. N.Y. Acad. Sci. 121*, 404–427.

Enomoto, T., Tanuma, S., & Yamada, M. (1983) *Biochemistry* (preceding paper in this issue).

Hachmann, H. J., & Lezius, A. G. (1975) *Eur. J. Biochem. 50*, 357–366.

Hesslewood, I. P., Holmes, A. M., Wakeling, W. F., & Johnston, I. R. (1978) *Eur. J. Biochem. 84*, 123–131.

Kornberg, A. (1980) *DNA Synthesis*, W. H. Freeman, San Francisco.

Lamothe, P., Baril, B., Chi, A., Lee, L., & Baril, E. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 4723–4727.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Matsukage, A., Sivarajan, M., & Wilson, S. H. (1976) *Biochemistry 15*, 5305–5314.

Nishioka, N., Matsukage, A., & Takahashi, T. (1977) *Cell Struct. Funct. 2*, 61–70.

Ohashi, M., Taguchi, T., & Ikegami, S. (1978) *Biochem. Biophys. Res. Commun. 82*, 1084–1090.

Ono, Y., Enomoto, T., Hanaoka, F., & Yamada, M. (1978) *Gann 69*, 207–212.

Ono, Y., Enomoto, T., & Yamada, M. (1979) *Gann 70*, 527–532.

PedraliNoy, G., & Weissbach, A. (1977) *Biochim. Biophys. Acta 477*, 70–83.

Seki, S., & Mueller, G. C. (1976) *Biochim. Biophys. Acta 435*, 236–250.

Tanuma, S., Enomoto, T., & Yamada, M. (1979) *J. Biol. Chem. 254*, 4960–4962.

Tanuma, S., Enomoto, T., & Yamada, M. (1980) *Cell Struct. Funct. 5*, 27–37.

Weissbach, A. (1977) *Annu. Rev. Biochem. 46*, 25–47.

Yoshida, S., Kondo, T., & Ando, T. (1974) *Biochim. Biophys. Acta 353*, 463–474.