Natural Science Report, Ochanomizu University, Vol. 45, No. 1, 1994 79
お茶の水女子大学 自然科学報告 第45巻 第1号

# Characterization of Nuclear, and Nuclear Matrix-Bound DNA Polymerases of Normal Rat Liver

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(Received Apr. 5, 1994)

## Summary

DNA polymerases in the nuclei and those bound to the nuclear matrices isolated from normal rat liver (at $G_0$ phase) were characterized. The nuclei from 1 gram tissue contained 0.46 unit of DNA polymerase $\alpha$ (0.05 U/mg protein) and 19 units of DNA polymerase $\beta$ (2.2 U/mg), but the nuclear matrices from 1 gram tissue had less than 0.01 unit of DNA polymerase $\alpha$ (2% of the activity in the nuclei) and 0.4 unit of DNA polymerase $\beta$ (2% of the nuclear activity). In contrast, regenerating rat liver (at S-phase) contained more than ten times higher activity of DNA polymerase $\alpha$ in the nuclei (5.2 U/g tissue) and 20% of the activity of this enzyme was bound to nuclear matrices. S-phase nuclei contained almost the same activity of DNA polymerase $\beta$ (21 units/g tissue) as that in the $G_0$ nuclei, and 0.5% of the $\beta$ activity was bound to the nuclear matrices. These findings indicate that no functional replication complex is assembled in the $G_0$ nuclei and no significant DNA polymerases are bound to the nuclear matrices.

**Key words:** nuclear matrix; DNA synthesis; rat liver

## Introduction

The nuclear matrices are known as the site of DNA replication in eukaryotic cells [1-5], and the multi-enzyme complexes including DNA polymerase $\alpha$ are associated with the matrices [6, 7]. The nuclear matrices isolated from rat regenerating liver were characterized and were shown to catalyze the formation of replication intermediates *in vitro* [8]. These findings show the existence of a putative DNA replication machinery including DNA polymerase $\alpha$ on the nuclear matrices in the actively replicating cells, and the machinery is considered to be assembled associated with the onset of DNA replication. But the characteristics of the nuclear matrices from resting cells ($G_0$-phase cells) are not understood to the detail, and the knowledge of the characteristics of $G_0$-matrics may give an important clue to resolve the problem on the assembly of the active

replication machinery.

In the present study, we characterized DNA polymerases and chemical composition of the nuclear matrices isolated from the normal rat liver (at $G_0$ phase). Our results show an absence of a replication complex on $G_0$-phase matrices, and the active complex may be assembled after the cells receive a growth stimulus.

## Materials and methods

*Isolation of nuclei and nuclear matrices.* Liver nuclei and nuclear matrices were isolated from either normal or two-third hepatectomized male rats (Donryu strain, 200–250 g) according to Berezney and Coffey [9, 10] with slight modifications.

Pieces of rat liver were homogenized in 20 vols. of solution A containing 0.4% Triton X-100 using a Teflon glass homogenizer. Solution A consisted of 0.25 M sucrose, 5 mM Tris-HCl (pH 7.0), and 5 mM $MgCl_2$. After addition of an equal volume of solution A, the homogenate was filtered through a nylon mesh (pore size: 26 $\mu$m), followed by centrifugation at $780\times g$ for 15 min. The precipitate was suspended in solution A containing 2.4 M sucrose and centrifuged at $30{,}000\times g$ for 90 min. The pellet was resuspended in solution A and washed three times, then the resultant precipitate was designated as the isolated nuclear fraction. The number of the nuclei was determined by counting in a hemocytometer.

Isolated nuclei suspended in solution A were mixed with pancreatic DNase I (1,000 units/ml) and incubated overnight at 0°C followed by centrifugation at $1{,}000\times g$ for 15 min. The pellets were then consecutively extracted twice with HS buffer consisted of 2.0 M NaCl, 0.2 mM $MgCl_2$, 10 mM Tris-HCl (pH 7.4). Centrifugation was done at $10{,}000\times g$ for 5 min at each time. The resultant pellet resuspended in solution A was designated as the isolated nuclear matrix.

*Assay of DNA polymerase activity.* The isolated nuclei or matrices ($4.0\times10^6$—$2.0\times10^7$) were incubated with a standard reaction mixture at 37°C for 10 min as described before [11] with a slight modification. The standard reaction mixture contained 25 mM Tris-HCl (pH 7.5), 7 mM $MgCl_2$, 20 $\mu$M each of dATP, dCTP and dGTP, 2.5 $\mu$M dTTP containing 2.0 $\mu$Ci [$^3$H]dTTP (96.8 Ci/mmol, New England Nuclear), 0.1 mg/ml activated calf thymus DNA, 2 mM 2-mercaptoethanol, 40 mM NaCl, 400 $\mu$g/ml DNase-free bovine serum albumin, 10% glycerol in a final volume of 25 $\mu$l. The DNA polymerase $\alpha$ activity was corrected for *N*-ethylmaleimide (NEM) resistant incorporation measured after preincubation of the sample with

10 mM NEM at 0°C for 30 min. For the assay of DNA polymerase $\beta$, samples were preincubated with 10 mM NEM as described above. After incubation, the reaction was stopped and the nuclei or the matrices were lyzed by adding 25 $\mu$l of 1.0% sodium dodecyl sulfate/100 mM Tris-HCl (pH 8.0)/0.5 M NaCl/50 mM EDTA/2 mM ATP. The lysate was transferred onto a disc of Whatmann 3MM filter paper and washed once with ice-cold 10% trichloroacetic acid (TCA) containing 2% sodium pyrophosphate for 15 min and three times with 5% TCA for 15 min and finally with 99% ethanol for 5 min. After drying, the radioactivity retained on the filter disc was counted in a toluene-based scintillator. One unit of enzyme activity is defined as the polymerization of 1 pmol of [$^3$H]dTTP in the 10 min assay.

The polymerase reaction was carried out at 37°C for 10 min because the reaction kinetics was linear for at least 20 min in all assays.

*Other methods.* DNA and RNA were separated according to Munro and Fleck [12], and DNA was determined by the method of Burton [13]. Protein determination was done according to Bradford [13], and RNA and phospholipid were analyzed as described by Berezney *et al.* [14].

## Results and Discussion

*Chemical composition of the nuclear matrix preparation from G-phase rat liver.*

DNase treatment and subsequent washing with high salt buffer (2.0 M NaCl) led to a considerable loss of DNA (85%) from the nuclei while 21% of protein in the nuclei still remained insoluble as shown in Table I. The matrix fraction consisted mainly of protein, the ratio of DNA to protein being 0.07, RNA to protein was less than 0.001, and phospholipid to protein was less than 0.001, respectively. The preparation consisted of three major polypeptides of 69, 66 and 62 kDa, as well as several minor polypeptides of 50 and >100 kDa, in SDS-polyacrylamide gel electrophoresis (data not shown). These are very similar to those reported before by Berezney and Coffey [15].

Table I. Chemical composition of the isolated nucleus and nuclear matrix.

| | Nucleus | Matrix |
|---|---|---|
| protein (pg) | 100 (100%) | 21 (21%) |
| DNA (pg) | 9.9 (100%) | 1.5 (15%) |
| RNA (pg) | 0.6 (100%) | 0.01 (1.7%) |
| phospholipid (pg) | 0.7 (100%) | 0.01 (1.4%) |

*Identification of DNA polymerases involved in DNA synthesis by the nuclear matrix.*

Effects of inhibitors on DNA synthesis catalyzed by $G_0$-nuclear matrix was studied to determine the type of DNA polymerase involved (Table II). Aphidicolin [16] and PHYLPA [17-20], which are specific inhibitors of DNA polymerase $\alpha$, inhibited almost all the $\alpha$ polymerase at less than 20 $\mu$g/ml and 40 $\mu$g/ml, respectively. 7% inhibition of the enzyme was obtained with about 20 $\mu$g/ml aphidicolin, and 8% inhibition with 40 $\mu$g/ml PHYLPA. 10% inhibition was observed with NEM, which is known to inhibit $\alpha$ polymerase, and 90% of the activity remained resistant, even in the presence of 20 mM NEM. ddTTP, an inhibitor of DNA polymerase $\beta$ [16], inhibited 87% activity of DNA synthesis in the nuclear matrix. These results suggest that about 10% of the DNA synthesis by the matrices are due to DNA polymerase $\alpha$, and about 90% of the activity are due to $\beta$ polymerase.

Table II. Effects of inhibitors on DNA synthesis catalyzed by $G_0$-nuclear matrix.

| Inhibitor | DNA synthesis (%) |
|---|---|
| None | 100 |
| Aphidicolin (20 $\mu$g/ml) | 93 |
| PHYLPA (40 $\mu$g/ml) | 92 |
| *N*-Ethylmaleimide (20 mM) | 90 |
| ddTTP (ddTTP/dTT=10) | 13 |

The DNA polymerase activities in the matrices and in the nuclei from normal rat liver ($G_0$ phase) are summarized in Table III. $G_0$-nuclear matrices isolated from 1 gram of the tissue bound 0.01 unit (U) of DNA polymerase $\alpha$ ($<0.01$ U/mg protein) and 0.4 U of DNA polymerase $\beta$ (0.3 U/mg protein). These values are correspond to about 2% each of the

Table III. DNA polymerase activities in isolated nuclei and nuclear matrices.

| | pol $\alpha$ | | pol $\beta$ | |
|---|---|---|---|---|
| | s.a.[1] (U/mg protein) | t.a.[2] (U/g tissue) | s.a. (U/mg protein) | t.a. (U/g tissue) |
| $G_0$-nuclei | 0.05 | 0.46 | 2.2 | 19 |
| $G_0$-matrices | <0.01 | 0.01 | 0.3 | 0.4 |
| S-nuclei | 0.7 | 5.2 | 2.8 | 21 |
| S-matrices | 1.5 | 1.0 | 0.1 | 0.1 |

[1]specific activity
[2]total activty

total activities present in the isolated $G_0$-nuclei. In contrast, S-phase nuclear matrices from 1 g tissue bound 1.0 U of DNA polymerase $\alpha$ (20% of the total activity in the nuclei) and 0.1 U of DNA polymerase $\beta$ (0.5% of the total). The values obtained in S-phase matrices and nuclei are consistent with the results obtained by other investigators [1].

These findings show that the absence of an active replication complex on $G_0$-phase matrices, and when the cells are stimulated for the growth, it may be assembled and constructed. Based on these results, the mechanism of an assembly of replication complex will be studied.

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