THE ROLE OF RAT LIVER NUCLEAR DNA POLYMERASE AND ITS DISTRIBUTION IN VARIOUS CLASSES OF LIVER NUCLEI

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1. Introduction

Rate zonal centrifugation has proved a useful technique for the separation of rat liver nuclei into various classes [1]. Studies of labelling *in vivo* of nuclei following injection of ^{3}H-thymidine and ^{14}C-orotic acid enable an identification of nuclei active in synthesis of DNA and RNA [2]. The distribution of a number of nuclear enzymes including RNA polymerase, NMN-adenylyl-transferase and polyADPR ligase have been investigated [2, 3] and certain significant correlations have come to light. One of the most important functions of the nucleus is the replication of its DNA, and DNA polymerase may be involved in this process. If nuclei are isolated from aqueous media only about 20% of the total DNA polymerase is recovered in the nuclear fraction, the greater part being in the cytosol [4], whereas a substantially greater proportion of the enzyme is found in nuclei prepared in non-aqueous media [5, 6]. In view of the continuing interest in mammalian DNA polymerase [7, 8, 9] we have investigated its distribution in fractionated rat liver nuclei and especially its relationship to the nuclei active *in vivo* in DNA synthesis. These experiments show that the specific activity of DNA polymerase is substantially reduced in nuclei with the maximal labelling.

2. Materials and methods

Wistar rats (120 g) were used for the preparation of nuclei as described elsewhere [1]. The method for zonal centrifugation [1] was modified by the use of an M.S.E. HS-A rotor in an M.S.E. High Speed 18 Centrifuge fitted with a speed controller. The gradient was 26–66% (w/w) glycerol containing 1 mM $MgCl_2$, 5 mM 2-mercaptoethanol and 5 mM tris HCl, pH 7.4. Centrifugation was carried out at 4°. The sample (10 ml) was loaded at 600 rpm and run at 1200 rpm for 15 min. For assay of DNA polymerase the effluent from the zonal rotor, which was unloaded at 600 rpm, was collected in fractions (25 ml) after passing through a flow cell. The nuclei were collected by centrifugation at 600 *g*, and resuspended in 50 mM tris-HCl, pH 7.5 containing 5 mM $MgCl_2$, 25 mM KCl and 1 mM 2-mercaptoethanol. It was established that the incorporation was linear with respect to time and concentration of nuclei for each fraction. Assays were done as in table 1.

Deoxyribonucleoside triphosphates and calf thymus DNA (Type 1) were obtained from the Sigma Chemical Company, and ^{3}H-TTP (13 Ci/mmole) and ^{3}H-thymidine (21.6 Ci/mmole) from the Radiochemical Centre, Amersham. Rat liver DNA was prepared from purified liver nuclei by a method similar to that of Gilmour and Paul [10].

3. Results

Some of the characteristics of DNA polymerase activity in unfractionated nuclei are shown in table 1. Activity is dependent on exogenous DNA, the native

Table 1
Characteristics of DNA polymerase activity in unfractionated rat liver nuclei.

Complete	100
-DNA	11
-Mg^{2+}	3
-dGTP, dCTP and dATP	55
Denatured instead of native DNA	47

Values are expressed as percentages. The complete system contained in a volume of 0.25 ml; tris-HCl, pH 7.5, 15 μmoles; $MgCl_2$, 2.5 μmoles; ATP, 0.5 μmoles; dATP, dGTP, dCTP and ^{3}H-TTP (45–90 μCi/μmole), 25 nmoles each; 2-mercaptoethanol, 0.25 μmole; native rat liver (or other) DNA, 50 μg; and 3–4 X 10^6 nuclei (186 μg protein). After incubation for 1 hr at 37°, the reaction was stopped with 0.5 ml ice-cold 0.1 M sodium pyrophosphate followed by 1 ml 17% trichloroacetic acid. The precipitate was collected by centrifugation, washed twice with 5% trichloroacetic acid and dissolved in 0.1 ml 98% formic acid and counted in a BBOT-containing liquid scintillation mixture at 25% efficiency. The complete system incorporated 480 pmoles ^{3}H-TMP per mg protein.

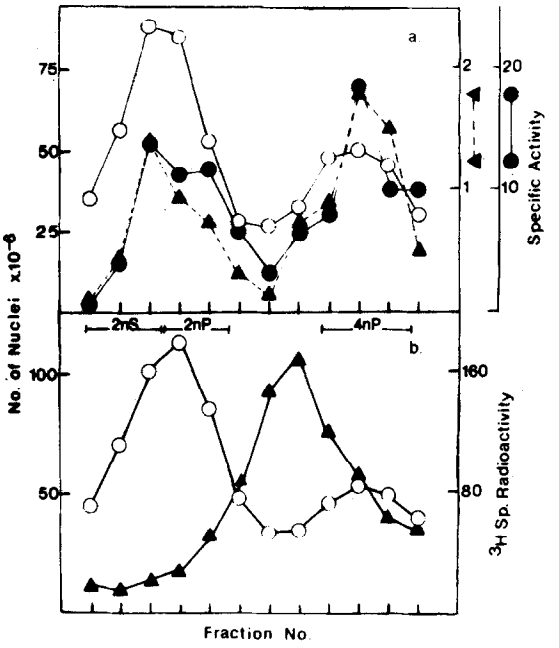

Fig. 1. (a) DNA polymerase specific activity (pmoles ^{3}H-TMP/10^6 nuclei) in rat liver nuclei separated by zonal centrifugation. Sedimentation is to the right. Assays were carried out as in table 1. o—o, nuclei per fraction; •—•, assayed with native rat liver DNA (curve A); ▲- - -▲, assayed without exogenous DNA (curve B).

(b) ^{3}H-Specific activity in nuclei following injection of ^{3}H-thymidine (25 μCi/animal) 1 hr before death. o—o, nuclei per fraction; ▲—▲, ^{3}H-specific activity (cpm/10^6 nuclei). 2nS, stromal diploid, 2nP and 4nP, parenchymal diploid and tetraploid nuclei.

template being preferred. Fig. 1a shows the distribution of enzyme activity among the different classes of nuclei following zonal fractionation. Fig. 1b shows the *in vivo* labelling pattern after a 1 hr pulse of ^{3}H-thymidine.

It is evident that the specific activity of DNA polymerase follows the nuclear profile (fig. 1a, curve A) and in several experiments activity appears to follow the parenchymal rather than the stromal nuclei. There is approximately twice as much activity in the tetraploids. Significantly the specific activity of DNA polymerase is lower in the regions of the gradient where DNA synthesis has occurred *in vivo*. The profile of incorporation by isolated nuclei is greatly reduced in the absence of exogenous template but nevertheless shows some resemblance to that with added template (fig. 1a, curve B). If the polymerase in nuclei labelled during *in vivo* DNA synthesis was in the form of an active complex, or at least in close association with endogenous DNA, the addition of triphosphates might have been expected to produce a peak of activity. However, a plot of the ratio of polymerase activity in the presence and absence of exogenous template (curve A/curve B), shows that the addition of exogenous DNA produces a relatively greater stimulation of activity in the region of *in vivo* DNA synthesis, than in the diploid and tetraploid peaks (fig. 2).

Results identical with those for curve A (fig. 1a) were also obtained when native calf thymus DNA was used as the template and the assay carried out using different assay conditions (glycine buffer, pH 8.5, shorter incubation times, and omitting ATP). In an experiment comparing native versus denatured calf thymus DNA, no change in template specificity could be found at any point across the gradient. We have also shown that plots of radioactive incorporation against substrate level for the nucleotides and for native rat liver DNA are identical for nuclei in the diploid peak and in the dip between the peaks. We have been unable to detect any substantial deoxyribo-

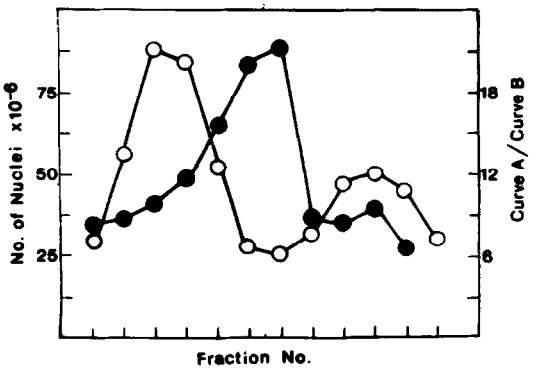

Fig. 2. Ratio of DNA polymerase specific activity assayed with and without exogenous rat liver DNA (curve A/curve B from fig. 1a). o—o, nuclei per fraction; •—•, curve A/curve B.

nuclease activity which, because it was present in variable amounts, might account for the apparent distribution of the DNA polymerase activity. Furthermore no appreciable enhancement of activity could be obtained by sonication of either unfractionated nuclei or of those from the various zonal fractions.

Experiments were also performed in which each zonal fraction was divided into two portions, one being assayed with a four, the other with a one triphosphate reaction mixture. The result (fig. 3) shows that in the region of *in vivo* DNA synthesis there is a relatively greater preference for a replication over a terminal addition reaction.

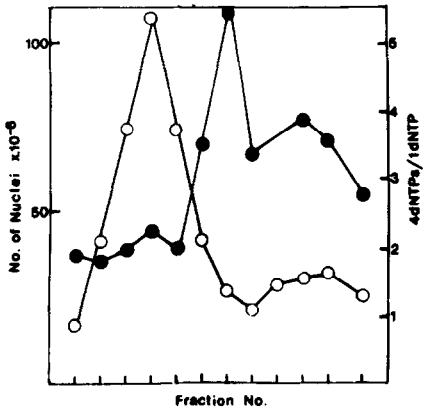

Fig. 3. Ratio of DNA polymerase specific activity with 4 deoxyribonucleoside triphosphates or 1 triphosphate (omitting dATP, dCTP, dGTP) in the reaction mixture. o—o, nuclei per fraction; •—• ratio of specific activities (4 dNTPs/1 dNTP).

4. Discussion

In the present studies we have been unable to detect more than small amounts of DNA polymerase in those nuclei which, because they are labelled *in vivo*, are known to have been active in DNA synthesis immediately prior to isolation. It is unlikely that this is due to inaccessibility of the enzyme in these nuclei since DNA polymerase activities are similar in intact and in sonically disrupted nuclei.

Although Friedman [7] has claimed a role for the nuclear polymerase in DNA replication, a correlation between the level of DNA polymerase in the soluble fraction and the *in vivo* rate of DNA synthesis, as measured by ^{3}H-thymidine labelling, has been established by others [11, 12]. This suggests that our results might be explained by the preferential loss of the replicative enzyme during aqueous isolation procedures. Alternatively, the active complex in nuclei engaged in DNA synthesis *in vivo* may be more labile than the enzyme from inactive nuclei. Irrespective of these possibilities, the residual activity of the nuclei in the dip nevertheless appears to have distinct properties (figs. 2 and 3).

Only 1–2% of nuclei in an adult rat liver are making DNA and these are concentrated on the zonal gradient in fractions where they constitute as much as 10–20% of the nuclei. However, these nuclei have a lower specific activity of DNA polymerase. Therefore, either the nuclear enzyme is not involved in replication of DNA, at least without further modification, or it has a repair function or there is a preferential loss or inactivation of the active enzyme as discussed above.

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