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Short Communication

Variable association of DNA polymerase with the cytoskeletal fractions of resting and dividing cells

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Abstract. A DNA polymerase activity associated with the detergent insoluble cytoskeletal fraction has been identified in dividing and non-dividing rat hepatocytes and a hepatoma (the Zajdela Ascitic Hepatoma). About 35 % of the enzyme is found associated with the cytoskeletal fraction of non-dividing cells as compared to about 3–6 % of the enzyme in dividing cells even though the dividing cells contain larger amounts of the extranuclear enzyme. The properties of the enzyme are similar to those of DNA polymerase-α. It is suggested that the association of the enzyme with the cytoskeletal fraction has functional significance.

Keywords. α-DNA polymerase; cytoskeleton; Zajdela Ascitic Hepatoma.

Introduction

The intracellular location of DNA polymerase-α, the major DNA replicating enzyme has been controversial (Sarngadharan *et alb.,* 1978). Recent immunocytochemical studies have indicated a predominantly nuclear location of the enzyme (Bensch *et al.,* 1982; Matsukage *et al.,* 1983; Nakamura *et al.,* 1984). But biochemical extraction procedures (Bollum and Potter, 1958; Keir, 1965) as well as certain immunofluorescence studies have indicated a large cytoplasmic pool (Brown *et al.,* 1981). There are, in addition, other reports indicating the association of the enzyme with cytoplasmic membrane (Baril *et al.,* 1970, 1971; Davis *et al.,* 1971). We have also reported a DNA polymerase-α like activity in the plasma membranes of a rat hepatoma, closely associated with the detergent insoluble fraction (Shashikant *et al.,* 1983).

A major discrepancy in presenting an exclusively nuclear location of the enzyme (Bensch *et al.,* 1982; Matsukage *et al.,* 1983; Nakamura *et al.,* 1984) arises from the fact that the site of synthesis of the enzyme is expected to be the cytoplasm. It is therefore surprising not to find the enzyme in the cytoplasm by immunocytochemical methods. We have now adopted a modified approach to address this question. Treatment of cells with a nonionic detergent solubilizes most of the cellular proteins (soluble fraction) leaving behind an insoluble material consisting of filamentous structure along with the nuclei. Intact nuclei can then be separated by double detergent treatment and low speed centrifugation. This supernatant is the cytoskeletal fraction (CSF) (Cervera *et al.,* 1981).

Abbreviations used: ZAH, Zajdela Ascitic Hepatoma; CSF, cytoskeletal fraction; PM, plasma membrane; PBS, phosphate buffered saline; NP 40, nonidet P 40; NEM, N-ethylmaleimide; PMSF, phenylmethylsulphonylfiuoride; SDS, sodium dodecyl sulphate.

We have examined the distribution of DNA polymerase-α in these fractions from resting and dividing rat liver cells and a rat hepatoma (Zajdela Ascitic Hepatoma, ZAH) cells.

Materials and methods

Chemicals

Radioactively labelled nucleoside triphosphates were obtained from Amersham, England, cold triphosphates and synthetic templates from PL Biochemicals. All other chemicals were from Sigma Chemical Company, St. Louis, Missouri, USA.

Cell suspension

Livers were obtained from both normal and partially hepatectomised adult Wistar rats (30–36 h after hepatectomy). Fetal livers were obtained from 18–19 days old embryos. After perfusion with cold phosphate buffered saline (PBS) (135 mM NaCl, 2·75mMKCl,1·5mMKH_2PO_4, 8 mM Na_2HPO_4, pH7·2), the tissues were minced and gently homogenized in PBS (10 ml/gm of tissue) in a loose fitting homogenizer. After passing the homogenate through anylon mesh (size 50–70 *μ)* it was centrifuged at 700*g* to pellet the cells. The pellets were resuspended in 10 mM PIPES buffer, pH 6·8, containing 10 mM KCl, 1 mM phenyl methyl sulphonyl fluoride (PMSF) and 10% sucrose (w/v).

Tumour cells

ZAH cells were obtained from the ascitic fluid from tumour bearing Wistar rats maintained by serial transplantation in the laboratory (Shashikant *et al.,* 1983). The cells were harvested from the peritoneal cavity of the animals between the 5th and 7th day of transplantation, diluted immediately with PBS and pelleted down at 700 *g* and were washed twice with PBS.

Sub cellular fractionation

Fractionations were performed according to the procedures already described (Cervera *et al.,* 1981). All operations were carried out at 4°C. Cells were resuspended at 2–4 × 10^6/ml in PIPES buffer and NP40 was added slowly to make the detergent concentration 1 % (v/v). The suspension was stirred for 15 min and then centrifuged at 1,000 *g* for 10 min. The supernatant (soluble fraction) was collected and preserved; the pellet was washed and resuspended in 1/3 ofthe original volume of buffer (10 mM Tris, pH 7·4, 10 mM NaCl, 1·5 mM $MgCl_2$, 5 mM $CaCl_2$ and 1 mM PMSF) and gently homogenised in a loose fitting homogenizer in the presence of 1 % Tween 40 and 0·5 % deoxycholate. The nuclei were pelleted by centrifugation at 750 *g* for 10 min. The nuclear pellets were routinely examined under the microscope to ensure that they were intact. The supernatant containing the CSF was centrifuged at 90,000 *g* for 90 min and the pellet obtained was taken up in 1 ml of Tris buffer. Plasma membranes (PM) were prepared using the method of Lesko *et al.* (1973) and PM skeleton was prepared as described earlier (Shashikant *et al.,* 1983).

Assays of DNA polymerase (Shashikant *et al.,* 1983) 5'-nucleotidase (Gurd and Evans, 1974) alkaline phosphatase (Pekarthy *et al.,* 1972) succinic dehydrogenase (Earl and Korner, 1965) and pyrophosphatase (Shatton *et al.,* 1981) were carried out according to the methods described earlier.

Protein concentrations were determined by the Lowry method (Lowry *et al.*,1951) in the presence of sodium dodecyl sulphate (SDS). Gel electrophoresis (Laemmli, 1970) and subsequent staining (Oakely *et al.,* 1980) were carried out as described elsewhere.

Results

Cytoskeletal fractions

Detergent treatment solubilizes about 70 % of protein which includes marker enzymes such as pyrophosphatase, alkaline phosphatase and succinic dehydrogenase. However, 5'-nucleotidase, an enzyme found in association with plasma membrane skeleton of liver cells (data not shown), was retained on the CSF indicating a certain commonality between PM skeleton and CSF. Electrophoretic analysis further revealed (figure 1) many common proteins between PM skeleton (lane 2) and CSF (lane 1) of liver except for proteins P 80, P 59, P 41 and P 40 which are specific to CSF. A similar comparison of ZAH-CSF (lane 3) with ZAH-PM skeleton (lane 4) shows protein P 120, P8 3, P77, P68 and P51 are specific to ZAH-CSF. However, PM skeleton of ZAH appears to have a distinct profile as compared to CSFs and PM skeleton of liver. A comparison of protein profiles of CSF fractions of liver and ZAH show distinct patterns with characteristic proteins in each cell type.

DNA polymerase activity in the soluble fraction and in CSF.

The DNA polymerase activities determined using activated calf thymus DNA as template from both soluble and CSF were inhibited by NEM. The percentage inhibition in the presence of 1 mM N-ethylmaleimide (NEM) varied between 82–87 % in the different samples tested. None of the samples examined showed any activity with synthetic polymers such as poly rA:oligo dT or poly rC:oligo dG or with a ribopolymeric template, AMV RNA as templates (results not shown). These properties suggest that the enzyme is likely to be DNA polymerase-α and also rule out contamination of DNA polymerising activities such as γ-polymerase and reverse transcriptase in the activity that we are assaying.

Distribution of DNA polymerase activity

The amounts of DNA polymerase activity from the different samples examined are shown in table 1. The total amount of enzyme present in the soluble and CSFs taken together is much higher in the dividing cells. Further, there is a dramatic difference between dividing and non-dividing cells in the distribution pattern of the enzyme in the soluble fraction and CSF. In the adult liver cells 35 % of the enzyme is found in CSF whilst the amount of enzyme present in this fraction of dividing cells is only 3–6 %. Even when the total amount of extranuclear enzyme present in adult liver cells is lower compared to that in dividing cells the absolute quantity of enzyme (per/g) found

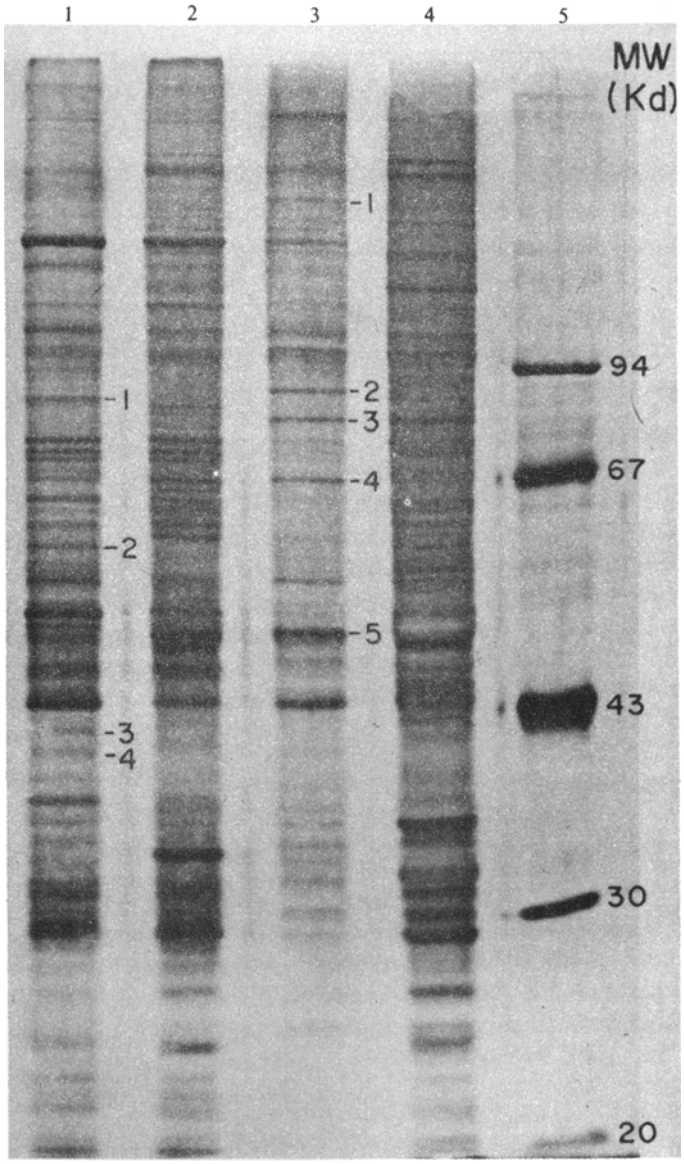

Figure 1. SDS Polyacrylamide gel electrophoresis (10%) profile of proteins from plasma membrane skeletons and CSF isolated from liver and ZAH cells. Lane 1, liver CSF; Lane 2, liver PM skeleton; Lane 3, ZAH CSF; Lane 4, ZAH PM skeleton; Lane 5, molecular weight markers (obtained from Pharmacia). Protein bands: Lane 1, bands 1,2,3 and 4 are P 80, P 59, P41 and P40 respectively. Lane 3: bands 1, 2, 3, 4 and 5 are P120, P83, P77, P68 and P51 respectively.

associated with CSF in these cells is larger than that present in the corresponding fractions obtained from diving cells. Table 1 also shows that there is considerable variation in the amount of enzyme present per mg of protein in the different samples. In CSFs this quantity is largest for adult liver cells.

Table 1. Extranuclear DNA polymerase activity in the soluble and cytoskeletal fractions of dividing and non-dividing cells.

	Activity in CSF + soluble fractions[a]						
	Per mg protein		in 1 gm tissue[b]		in 10^8 cells[c]		% (of CSF + cytoplasmic) activity in CSF
Sample source	sol[d]	CSF[d]	sol	CSF	sol	CSF	
Adult liver	1·9	6·5	131·1	69·0	437·1	230·9	34·5
Regenerating liver	6·5	2·8	375·9	22·7	1253·3	75·5	5·6
Fetal liver	11·2	1·8	670·0	33·0	670·0	33·0	4·7
ZAH cells	18·1	2·9	1740·0	54·0	870·3	28·6	3·1

[a] Activity expressed as *p* mol of [^{3}H]-TMP incorporated in 15 min. reaction using activated calf thymus DNA as template. Values reported are averages of 3 separate determinations in each case.
[b] Calucated from net weight of starting tissue.
[c] Adult liver and regenerating liver have 3×10^7 cells/g tissue whilst liver has 1×10^8 cells /gm tissue and ZAH has 2×10^8 cells/g tissue.
[d] sol, .soluble fraction.

Discussion

We have demonstrated biochemically the presence and characteristic distribution of DNA polymerase-α in the soluble and CSFs of resting and dividing cells. These observations are in sharp contrast with most immunocytochemical studies indicating an exclusive nuclear location for DNA polymerase-α. As the enzyme must be synthesized in the cytoplasm, it is surprising that no enzyme can be detected at the site of synthesis by immunocytochemical methods. It is therefore likely that these methods especially those making use of monoclonal antibodies may be detecting specific epitopes, which may be masked in the enzyme present outside the nucleus.

Differences in the state of association of DNA polymerase-α with nuclear matrix in resting and dividing cells have been documented (Smith and Berenzney, 1982). In the present experiments we demonstrate a differential association of the enzyme with CSF in resting and dividing cells. Such an association and possibly a functional role of the association is not surprising in light of the increasing realization of the role of CSF in a variety of cellular events. It is likely that the cytoskeletal framework may play a role in the transport of DNA polymerase-α within the cell. When the cells are dividing, any transport of the enzyme through the mediation of the skeletal framework could be more active and the steady state concentration of the amount of enzyme remaining associated with CSF could be lower. In the resting phase, part of the enzyme synthesized at the time of division could remain associated with skeletal elements probably better protected, to be readily available at the onset of cell division.

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