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DNA polymerase alpha from the nuclear matrix of cells infected with simian virus 40

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ABSTRACT

The nuclear matrix prepared from normal, simian virus 40 (SV40)-infected, and SV40-transformed cells contained DNA polymerase activities. Approximately 12% of the total DNA polymerase activities in isolated nuclei remained with the nuclear matrix. α-polymerase was the major matrix DNA polymerase activity as judged by sensitivity to various inhibitors: aphidicolin, dideoxy-TTP, and N-ethylmaleimide. Approximately 2-4 fold higher DNA polymerase activity was detected in matrices obtained from lytically infected and virus-transformed cells than that found in normal cells. In lytically infected cells, 30-50% of the matrix-bound DNA polymerase activity solubilized by sonication co-sedimented with majority of the matrix T-antigen, and was co-precipitated with anti-T sera. The results suggest that α-polymerase and viral T-antigen may form a functional complex in the matrix.

INTRODUCTION

Eucaryotic chromosome structure and synthesis have been extensively studied (1, 2, 3); however, little is known about the mechanism of how chromosome replication initiates in the nucleus. Recently, accumulated evidence of a tight association of mammalian DNA with the nuclear matrix has suggested that the nuclear matrix may provide fixed sites for attachment of DNA replication complexes (4,5). The nuclear matrix is experimentally defined as the residual proteinaceous structure obtained after treatment of isolated nuclei with detergent, followed by washing with hypotonic and hypertonic buffer (6, 7). The integrity of the matrix is maintained after exhaustive digestion with RNase and DNase (8, 9).

The large size and complexity of mammalian chromosomes makes it difficult to assess which of the replication steps, i.e. initiation, elongation or termination, can occur on the matrix. Small DNA tumor viruses, such as simian virus 40 (SV40) and polyoma, have been used as experimental models to elucidate the role of the nuclear matrix on either DNA replication or transcription (10, 11). Viral DNA replicates exclusively in the cell nucleus and complexes with cellular histones to form nucleosome structures (12, 13, 14). Viral

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specific DNA and the tumor (T) antigen of polyoma have been shown to associate with the nuclear matrix isolated from lytically infected (10) and transformed cells (15). But, the roles of the matrix in DNA synthesis are still not clear.

In this study, we demonstrated that enhanced DNA polymerase activities were associated with the matrix from both SV40 lytically infected and transformed cells. The major polymerase activity appeared to be α-polymerase. The results suggest that the matrix-bound DNA polymerase may either complex with viral T antigen or reside on the same network containing T antigen.

MATERIALS AND METHODS

Virus and cells

African monkey kidney cells (CV-1) were obtained from Dr. M. DePamphilis. Fisher rat fibroblasts (F111) and SV40 transformed F111 cells were provided by Dr. T. Benjamin. SV40 transformed embryonic hamster cells (THE-2) were obtained from Dr. K. Rundell. Cells were grown in Dulbecco's modified Eagles medium (DMEM) (KC Biological, Inc.) supplemented with 5% fetal calf serum (Gibco) and 0.5 ug/ml of gentamycin (Sigma) in a CO_2 incubator at 37°C. Simian virus 40 (SV40) stock was routinely prepared by infecting confluent CV-1 cultures with a multiplicity of infection (m.o.i.) of 0.005 and harvested by three cycles of freezing-thawing as described previously (16). For all the experiments described, confluent cultures were infected with SV40 at a m.o.i. of 50.

Isolation of nuclear matrix

Confluent monolayers (15 x 100 cm) of cells (normal, infected, and transformed) were washed and lysed in hypotonic buffer containing 10 mM Hepes, pH 7.8, 5 mM KCl, 0.5 mM $MgCl_2$, 0.5 mM dithiothreitol (DTT), 0.3 mM phenylmethylsulfonyl fluoride (PMSF), with a Dounce homogenizer with three strokes of a tight-fitting pestle. Nuclei were purified following the method of DePamphilis and Berg (17). The nuclear matrix was prepared from the purified nuclei by a modified method of Buckler-White et al. (10). The nuclear pellet was washed once in 10 mM Hepes, pH 7.8, 1 mM $MgCl_2$, 0.5 mM DTT, 50 mM KCl, 0.25 M sucrose, and suspended in TMS buffer containing 50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 0.25 M sucrose. Triton X-100 was added to a final concentration of 1% and the suspension was centrifuged at 500 x g for 10 minutes. These detergent-treated nuclei were resuspended in TMS buffer and incubated with DNase (50 ug/ml) and RNase (20 ug/ml) for 60 minutes at 37°C. The digested nuclear suspension was then pelleted and washed three times in LS buffer containing

10 mM Tris-HCl, pH 7.4, 0.2 mM $MgCl_2$, at 500 x g for 10 minutes. The nuclear pellet was subsequently extracted twice in HS buffer containing 10 mM Tris-HCl, pH 7.4, 2 M NaCl, 0.2 mM $MgCl_2$, on ice for 15 minutes and sedimented at 500 x g for 15 minutes. The final nuclear material was washed once in hypotonic buffer to remove the salt and resuspended in 100 ul of hypotonic buffer. The final preparation is designated as the nuclear matrix preparation. A final concentration of 0.3 mM PMSF was included in the buffers used throughout the experiments.

Preparation of radioactive nuclear matrix

At 36 hours after infection, confluent CV-1 monolayers were replaced with phosphate (Pi) free medium supplemented with 2% dialyzed fetal calf serum and 5 ug/ml of gentamycin. At 38 hours post-infection, the medium was decanted and cultures were replenished with the same medium containing 250 uCi/ml of carrier free ^{32}Pi for four hours. The nuclear matrix was then prepared by the procedures described above. The final matrix pellet was washed once with hypotonic buffer and directly solubilized by addition of 200 ul of disruption buffer which contained a 10 mM Tris-HCl, pH 7.6, 15 mM NaCl, 1% Nonidet P-40 (NP-40), 0.1% sodium deoxycholate, 0.1% sodium dodecylsulfate (SDS), 0.3 mM PMSF, and 0.5 mM DTT. This was followed by a brief sonication for 30 seconds by a cell disruptor (Kontes) at a setting of 3.5. Generally, 50-65% of the matrix components were solubilized, judged by the released ^{32}P radioactivity after centrifugation at 16,000 x g for 10 minutes.

DNA polymerase assays

DNA polymerase activities on the matrix were determined by the amount of α-^{32}P-dCMP incorporated into active salmon sperm DNA within 30 minutes at 37°C. The rate of synthesis was linear for at least 30 minutes under these conditions.

Total DNA polymerase activity was assayed in a 100 ul reaction mixture containing 2 mM ATP; 100 uM each of dTTP, dGTP, dATP, 5 mM phosphoenol pyruvate, 30 ug pyruvate kinase per ml, 10 ug of activated salmon sperm DNA (18), and α-^{32}P-dCTP (4,400 cpm/pmole). α-^{32}P-dCTP with a specific activity of 50-100 Ci/mmole was routinely prepared by the method of Johnson and Walseth (19). In reactions containing dideoxy-TTP, $MgCl_2$ was added to maintain the free Mg concentration.

α-polymerase activity was assayed in the same reaction mixture with an addition of aphidicolin at 1 mM. The difference of activity measured in the presence and absence of aphidicolin was designated as α-polymerase activity.

β-polymerase activity was determined in a reaction mixture containing 0.1

M KCl at pH 8.5, as described by Hübscher et al. (22). In some experiments, β-polymerase was determined in an assay similar to the α-polymerase. The nuclear matrix was always preincubated with 5 mM N-ethylmaleimide (NEM) at 0°C for 30 minutes.

γ-polymerase activity was determined according to the method of Knopt et. al. (23) in the presence of poly (rA)·$(dT)_{12}$ (50 ug/ml) (Boehringer Mannheim) as template-primer and ^{3}H-TTP (3,600 cpm/pmole).

Sucrose gradient analysis of matrix-bound DNA polymerases

The nuclear matrices (non-radioactive and ^{32}P-labelled) prepared from SV40 infected CV-1 cells were solubilized as described above and layered on a 5-40% linear sucrose gradient in 10 mM Tris-HCl, pH 7.4, 0.14 M NaCl, 1 mM DTT and 0.3 mM PMSF. The linearity of the sucrose gradients was calibrated by measuring the refractive index at 20°C with a refractometer (Bausch and Lomb). The gradients were centrifuged at 18,000 rpm for 12 hours at 4°C in a SW 55 rotor. Gradients were fractionated from the bottom of the tube. α-polymerase activity in each fraction was measured in the reaction mixture as described above. DNA polymerase activity in crude cytoplasmic fraction prepared from SV40 infected cells (24) was analyzed by a similar procedure. The labelled precursor used for the DNA polymerase assays was either α-^{32}P-dCTP (4,400 cpm/pmole) or ^{3}H-TTP (3,600 cpm/pmole).

Immunoprecipitation and gel electrophoresis

Each fraction (250 ul) collected from the sucrose gradient was adjusted to 10 mM Tris-HCl, pH 7.6, 1.2 M NaCl, 20 mM EDTA, 0.6% SDS, and incubated at 37°C for one hour. Each sample was then diluted to a final volume of 1 ml in buffer containing 0.15 M NaCl, 50 mM Tris-HCl, pH 7.4, 5 mM EDTA, 0.2% NaN_3, 0.2% bovine serum albumin, 10 mM DTT, and precipitated by anti-T sera following the method of Gurney et al. (25). The anti-T sera was prepared from tumor bearing hamsters which had been injected with SV40 transformed hamster cells, THE-2. The antigen-antibody complexes were then precipitated with the addition of 100 ul of 10% suspension of formalin fixed Staphyloccocus aereus Cowan I (26). The bacteria cells were then pelleted and washed twice with buffer containing 50 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 5 mM EDTA, 10 mM DTT, bovine serum albumin (1 mg/ml), NaN_3 (0.02%) and gelatin (0.2%) in an Eppendorf microfuge for 2 minutes. The washed bacteria cells were resuspended in hypotonic buffer containing 20 mM DTT, 1.5% SDS, and heated at 100°C for 3 minutes. The bacteria cells were then removed by centrifugation at 16,000 rpm for 2 minutes in a microfuge (Brinkman). The supernatant contained the eluted antigen-antibody complexes.

The eluted proteins were analyzed by gradient polyacrylamide gel electrophoresis using a slab gel 27 by 15 by 0.15 cm. A 7.5% to 15% linear gradient was prepared in the SDS-Tris-glycine system of Laemmli (27). The ratio of acrylamide to bisacrylamide was 36.5:1. Electrophoresis was carried out at 15 mA for 16 hours at room temperature. Gels were stained with Coomassie Blue, destained in acetic acid: methanol (7%.5%), and dried with a gel dryer (BioRad) Autoradiography of dried gel was carried out using Kodak XRP film and DuPont HI-plus intensifier screen at 70°C.

RESULTS

Association of DNA polymerase activity with the nuclear matrix

The DNA polymerase activity in isolated nuclei from normal and virus-infected cells can catalyze DNA synthesis *in vitro* (2, 28). To determine the distribution of DNA polymerase activity in the different fractions during the preparation of the nuclear matrix, DNA polymerase activities from cell lysate, purified nuclei, detergent-treated nuclei, DNase-treated nuclear complexes, or salt-washed nuclear complexes were measured with endogenous or exogenous template. Since the nuclear matrix prepared from nuclei in the presence of DNase I contained less than 0.01% of the total nuclear DNA, DNA polymerase activity associated with matrix was determined with an exogenous template, activated salmon sperm DNA. The results (Table 1) obtained from exogenous template demonstrate that approximately 35% of the polymerase activity in isolated nuclei was removed after treatment with Triton X-100. Seventy percent of the polymerase activity in nuclei was removed by the treatment with nuclease and repeated washing with LS buffer. The residual 12% of the DNA polymerase activity in isolated nuclei remained essentially intact on the nuclear matrix preparation during washing in HS buffer. Approximately 90% of the nuclear proteins were removed during the preparation of the nuclear matrix. Therefore, it was not surprising to find an increased activity (unit/mg protein) in the matrix (Table 1).

To eliminate the possibility that DNA polymerase activity found on the matrix was the result of nonspecific binding of the cellular DNA polymerase to matrix components, cytosol containing high polymerase activity (50 pmole/ug DNA) (24) was included during various stages of matrix preparation. The polymerase activity associated with the matrix (approximate 2.2×10^5 nuclei) was essentially the same, having approximately 0.02 pmole per ug of exogenous DNA template (data not shown). This result strongly suggested that soluble DNA polymerase did not bind nonspecifically to the matrix. Therefore, it is

Table 1. DNA polymerase activities in cell lysate, nuclei, and nuclear matrix

Preparation[a]	protein[b] content	DNA polymerase actvity			
		Endogenous and Exogenous template[c]	Endogenous template		Exogenous[d] template	
	(ug)	(pmole)	(pmole/10^6 nuclei)	(unit[e]/mg protein)	(pmole/ug DNA)	(unit[e]/mg protein)
Cell lysate	364	41.84	16.94	46.4	2.49	68.4
Isolated nuclei	172	17.56	9.66	56.1	0.79	45.9
Detergent-treated nuclei	112	10.78	5.58	49.7	0.52	46.4
LS nuclear complex	67	3.78	1.38	20.5	0.24	35.8
HS nuclear complex	19	1.07	0.07	3.6	0.10	52.6

[a]Different preparations were obtained from SV40 infected CV-1 cells (10^6 cells) as described in Materials and Methods

[b]Protein contents were determined by the method of Bradford (21).

[c]Nuclear DNA remaining associated with the nuclei was used as the endogenous template. DNA polymerase activity was measured in the reaction mixture containing both endogenous template and 10 ug of activated salmon sperm DNA which served as the exogenous template. The values represent the total activities.

[d]The values represent the net activities since they were corrected for endogenous activities.

[e]A unit is defined as one pmole of total dCMP incorporation into acid-insoluble material in 30 min. at 37 C.

very likely that DNA polymerase tightly associated with the matrix is an integral component of the matrix.

When the nuclear matrix was prepared from nuclei without the treatment with DNase, approximately 20% of nuclear DNA remained associated with the matrix. Limited DNA synthesized from this endogenous template by the matrix DNA polymerase could hybridize to both SV40 and cellular DNA. A small fraction (10%) of viral DNA synthesized in matrix migrated as relaxed circular SV40 DNA on an agarose gel (data not shown). The majority of the DNA synthesized in vitro appeared to be short fragments with sedimentation values of 4-5 S in neutral sucrose gradient (24). When the nuclear matrix was directly analyzed on a 5-30% sucrose gradient (24), matrix-bound DNA was mainly found near the bottom of the gradient. SV40 DNA sedimenting around 21 S was detected by filter hybridization (10) when the nuclear matrix was treated with protease prior to the sucrose gradient centrifugation. It is possible that viral DNA template associated with the matrix was also complexed with proteins as forms of nucleoprotein complexes or immature virion (10).

Type of DNA polymerase

In mammalian cells, both α-and β-polymerase are found in the nuclear fraction (29). To determine the type of DNA polymerase (i.e., α-, β-, or γ-polymerases) on the matrix prepared from SV40 infected CV-1 cells, polymerase activity was assayed in the reaction mixture including aphidicoln, dideoxy-TTP, NEM or poly (rA)·$(dT)_{12}$ as described in Materials and Methods.

Approximately 95% of the matrix DNA polymerase activities was inhibited by a specific inhibitor for α-polymerase, aphidicoln (1 mM) (30), and a non-specific inhibitor, such as NEM (1 mM) (Fig. 1, Table 2). Both β- and γ-polymerase activities were measured in purified nuclei treated with or without detergent (Table 2). After the subsequent treatment of nuclei with nuclease and 2 M NaCl, little or no γ-polymerase was detected (Table 2). Nevertheless, approximately 5% of β-polymerase remained associated with the nuclear matrix. This was in agreement with the observation that more than 90% of the polymerase activity on the matrix was resistant to dideoxy-TTP, an inhibitor for both β- and γ-polymerase (Fig. 1) (31). Similar sensitivities of matrix-bound DNA polymerases to the different inhibitors were also observed on matrices prepared from normal and virus-transformed cells (Table 3). All of these results strongly suggest that matrix-bound polymerases were primarily α-polymerase.

Polymerase activities of lytically infected and viral transformed cells were approximately 2-4 fold higher than the activity found in the normal cells (Table 3). Whether this enhanced activity reflected the stimulation of cell-

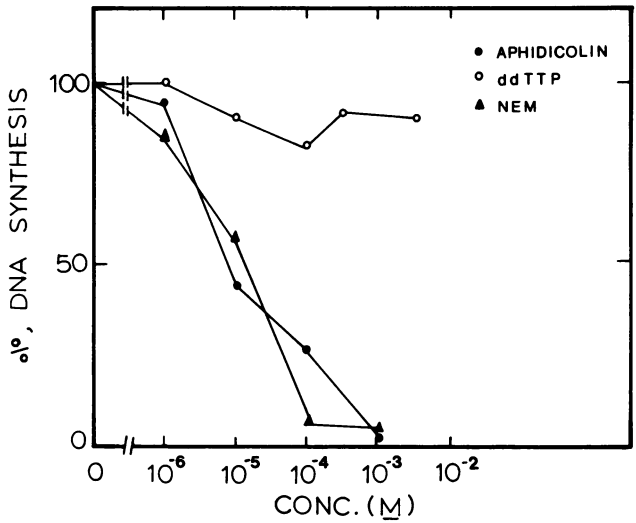

Figure 1. The effect of inhibitors on the matrix-bound DNA polymerase activity prepared from lytically infected cells.
DNA polymerase activity in the nuclear matrix prepared from SV40 infected cells (10^7 cells) was determined from the activated salmon sperm DNA (10 ug) as described in Materials and Methods. Various concentrations of aphidicolin, NEM, or dideoxy-TTP were included in each of the reaction mixtures.

ular DNA synthesis after infection with SV40, or the involvement of the matrix in viral DNA replication remains to be determined.

Matrix complexes containing T-antigen and DNA polymerase

To understand the functional significance of the matrix DNA polymerase, the matrix was solubilized by sonication, and fractionated by sucrose gradient centrifugation. At least two peaks of DNA polymerase activity were detected with activated salmon sperm DNA template (Fig. 3A). Approximately 30-40% of the polymerase activity was found in complexes around 23 S. In a parallel experiment, solubilized ^{32}P nuclear matrix obtained from infected cells was fractionated in the same manner, and immunoprecipitated with anti-T sera. Anti-T sera were shown to react specifically with SV40 T antigen (Fig. 2). Phosphorylated viral T-antigen was found in every fraction of the solubilized matrix (Fig. 3B). Most of the matrix T-antigen appeared to co-sediment with matrix-bound DNA polymerase activity (Fig. 3A, 3B). Both phosphorylated 56K nonviral T-antigen and possibly small t antigen (21k) were also detected in the immunoprecipitant of the matrix complex. The complexes containing T-antigen and DNA polymerase were soluble after centrifugation at 100,000 xg for 60 min. When the solubilized mixture was immunoprecipitated with anti-T sera prior to the gradient analysis, DNA polymerase activity normally found in the

Table 2. Distribution of α-, β-, and γ-polymerase activities in cell lysate, nuclei, and nuclear matrix

Preparation (10^6 cells)	Polymerase activity[a,b], (pmole x 10^{-2}/ug DNA)				Polymerase[b], (%)		
	Total Polymerase	aphidicolin (1 mM)	NEM (5 mM)	Poly(rA)·$(dT)_{12}$	α-,	β-,	γ-,
Cell lysate	249	82.5	25.3	18.1	79.4	12.0	8.5
Isolated nuclei	79	18.1	33.5	10.6	58.2	32.2	10.1
Detergent-treated nuclei	52	8.5	26.9	9.3	55.0	33.7	11.2
LS nuclear complex	24	2.2	0.4	0.3	83.8	14.9	1.1
HS nuclear complex	10	0.3	0.5	0.1	94.2	4.8	0.9

[a]The values represent the activities obtained from the exogenous template as described in Materials and Methods. The activity was measured in the reaction mixture in the presence and absence of exogenous template. Activated salmon sperm DNA (10 ug) was used in assays for α- and β-polymerase. Poly(rA)·$(dT)_{12}$ (5 ug) was used to determine γ-polymerase activity.

[b]Total polymerase activity was measured in the absence of inhibitors. The difference of activity obtained from the reaction in the absence (total polymerase) and presence of aphidicolin was designated as α-polymerase. The activity measured from a reaction mixture containing either NEM or poly(rA)·$(dT)_{12}$ represents directly the β- or γ-polymerase.

Table 3. Inhibition of matrix-bound DNA polymerase activities[a]

Nuclear Matrix Prepared	DNA synthesis (pmole/mg protein)[b]			
	Control	Aphidicolin (10^{-3} M)	NEM (10^{-3} M)	Dideoxy-TTP (10:1, ddTTP:TTP)
SV40 CV-1	96.57	1.93	4.44	88.6
CV-1	22.81	0.44	2.26	20.3
SV40 F111	21.07	1.1	1.68	18.4
F111	9.69	0.29	0.48	8.7

[a]DNA polymerase activity in nuclear matrices prepared from 8 plates (15 x 100 cm) of confluent normal CV-1, SV40 infected CV-1, normal F111, and SV40 transformed F111, were measured in the reaction mixture in the presence of 10 ug of salmon sperm DNA to 10 uCi of α-^{32}P-dCTP. Aphidicolin, NEM, or dideoxy-TTP was added to a final concentration as indicated in the reaction mixture.

[b]Since a monolayer (15 x 100 cm) of F111 cells has a significantly less number of cells than CV-1 cells, the activities were normalized by the amount of matrix protein added to each of the reactions. Protein content was determined by the method of Lowry et al. (20).

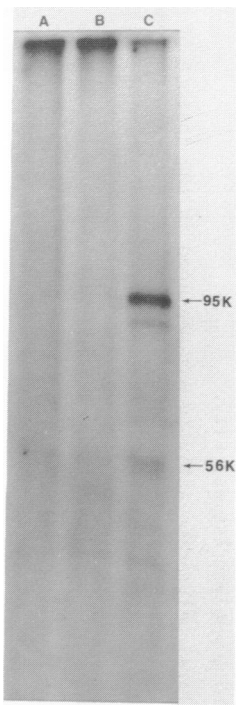

Figure 2. Immunoprecipitation of the nuclear matrices by anti-T sera.
Anti-T sera collected from tumor bearing hamsters were used to react with solubilized ^{32}P nuclear matrices prepared from normal (B) and SV40 infected (C) CV-1 cells. The immunoprecipitants were analyzed on a polyacrylamide gel as described in Materials and Methods. The nuclear matrix of infected CV-1 cells reacted with the normal serum (A) was used as a control. The autoradiography was carried out at -70°C.

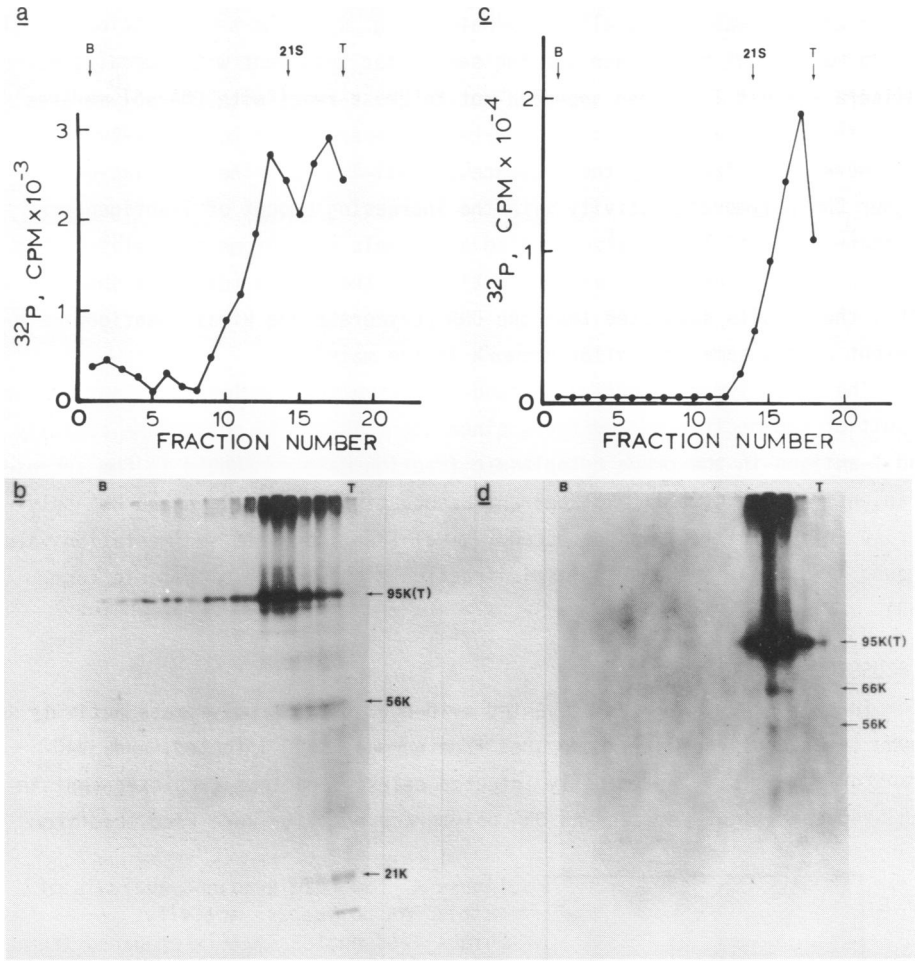

Figure 3. Sucrose gradient analysis of the nuclear matrix and cytoplasmic complex containing SV40 T-antigen and DNA polymerase activity. Both non-radioactive and radioactively labelled (^{32}P) nuclear matrices were prepared from SV40 infected CV-1 cells. Matrices were then solubilized and analyzed on a 5-40% linear sucrose gradient as described in Materials and Methods. (a) matrix DNA polymerase activity in unlabelled nuclear matrix. Each fraction was assayed for DNA polymerase in the reaction mixture containing 10 ug of activated salmon sperm DNA and 5 uCi of α-^{32}P-dCTP. (b) ^{32}P labelled matrix T-antigen. Solubilized ^{32}P matrix was fractionated on a 5-40% sucrose gradient, precipitated with anti-T sera, and analyzed on a 7.5-15% linear gradient of polyacrylamide gel as described in Materials and Methods. Fractions on the polyacrylamide gel corresponded to the fractions in the sucrose gradient. (c) Cytoplasmic DNA polymerase activity. Cytosol prepared from infected CV-1 cells (24) was fractionated in a sucrose gradient and DNA polymerase activity of each fraction was measured in a same manner as in (a). (d) Cytoplasmic T-antigen. ^{32}P cytosol was fractionated, immunoprecipitated and analyzed by gel electrophoresis as in (b). Sedimentation was from right to left.

matrix complex was essentially eliminated (Fig. 4). The sedimentation profile of the matrix DNA polymerase was the same after treatment with normal sera. Antisera against T-antigen appeared not to cross-react with DNA polymerases since the polymerase activities in cytosol prepared from normal CV-1 cells (24) were not affected by the presence of anti-T sera. The correlation of higher DNA polymerase activity with the increasing amount of T-antigen precipitated by anti-T sera also implied a possible stoichiometric relationship between DNA polymerase and viral T-antigen in the matrix (data not shown). Thus, the results suggested that the DNA polymerase and viral T-antigen were present on the same or similar network in the matrix.

The DNA polymerase activity found in the matrix complex could not be the result of nonspecific aggregation, since the bulk of DNA polymerase activity and T-antigen in the crude cytoplasmic fraction were separated in the sucrose gradients (Fig. 3C, 3D). No apparent association of T-antigen and DNA polymerase in cytosol (24) was detected. Viral T-antigen with sedimentation value around 18 S seen in the cytoplasmic fraction might be the oligomeric form (32).

DISCUSSION

In this study, we have presented evidence that DNA polymerase activity is found in the nuclear matrix prepared from normal, SV40-infected, and SV40-transformed cells. In lytically infected cells, tenacious complexes containing SV40 specific T-antigen and DNA polymerase activity were recovered from

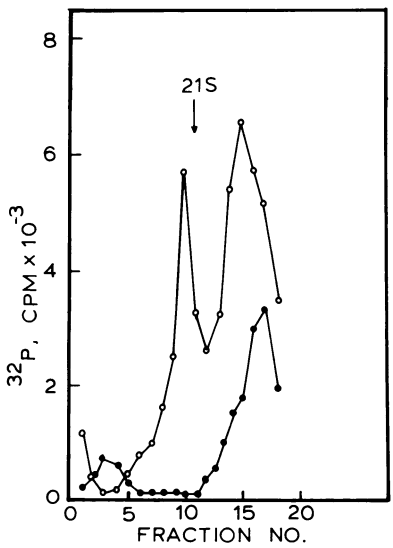

Figure 4. Sucrose gradient analysis of matrix DNA polymerase activity. Solubilized nuclear matrix prepared from SV40 infected cells was either directly loaded on a 5-40% linear sucrose gradient or precipitated with anti-T sera prior to the sucrose gradient analysis as described in Materials and Methods. Gradients were fractionated through the bottom of the tubes. DNA polymerase activity in each fraction was assayed. Symbols: o—o, matrix without the treatment of anti-T sera; ●—●, matrix treated with anti-T sera.

the solubilized nuclear matrix. The results support the concept that subnuclear structures are involved in the regulation of replication (4, 5).

The correlation between increased levels of α-polymerase activity with the onset of DNA synthesis in normal or infected cells as well as the effect of different inhibitors on DNA synthesis in vivo and in vitro, demonstrate that α-polymerase is responsible for replication, not repair synthesis (2). The nuclear α-polymerase has recently been shown to be both structurally and functionally similar to the cytoplasmic α-polymerase (33). The exact distribution of α-polymerase in the cell has not yet been resolved. The recent demonstration of the perinuclear location of α-polymerase by cytological and immunological methods (34) has implied that small amounts of α-polymerase may either be transported into the nucleus or become tightly associated with the nuclear matrix during DNA synthesis. Our finding that α-polymerase was associated with the nuclear matrix of various cells (Table 3) appears to support this hypothesis.

The nuclear matrix prepared from cells consisted mainly of proteins since proteases removed most proteins, as determined by gel electrophoresis, and completely eliminated α-polymerase activity. Matrix-bound DNA α-polymerase appeared to be active even after washing with 1% Triton X-100, 2 M NaCl and 0.01% SDS. The finding that DNA synthesis could continue on isolated matrix material in the absence of exogenous DNA suggested that endogenous replicating DNA is also associated with the matrix. So far, we were not able to show preferential SV40 DNA sequences associated with the matrix prepared from lytically infected cells, although it has been reported that SV40 specific DNA sequences are enriched in the nuclear matrix from SV40-transformed cells (15).

The matrix-bound DNA polymerase activity in SV40 lytically infected or transformed cells was significantly higher than that of its normal counterpart. The enhanced polymerase activity may be related to the increased numbers of either cellular or viral replicons since virus infection is known to induce cells passing through a normal DNA synthesis (S) phase (35, 36). Similar observations of enhanced polymerase activity has also been seen in the nuclear matrix from regenerated rat liver (37). Whether the increase in the matrix DNA polymerase activity is due to an increase in the numbers of polymerase molecules or the stimulation by other matrix components is not known. Several stimulatory factors for DNA polymerase have been reported in different cell systems (2). A nuclear DNA/T-antigen complex prepared from the sonicated nuclei have SV40 transformed cells has recently been reported to stimulate DNA polymerase activity (38). But, it is not clear whether or not the character-

istics of this complex is just reflecting the random shearing of the nuclear chromosomes, since the stimulatory effect can be removed after the treatment of this complex with DNase.

The finding of the matrix complex containing both T-antigen and DNA polymerase activity is very intriguing since SV40 T-antigen binds specifically to the SV40 origin of replication (39), and is required for viral DNA initiation (40, 41). The exact amount of viral T-antigen in the matrix was too low to be detected by Coomassie blue staining. However, judging from the amount of phosphorylated viral T-antigen precipitated by anti-T sera and subsequently separated by gel electrophoresis, approximately 11% of the total phosphorylated viral T-antigen remained with the nuclear matrix (Jones and Su, unpublished results). At present, we do not know what mediates the complex formation. It is unlikely that the DNA polymerase is directly associated with the T-antigen since viral T-antigen was found in every fraction of the gradient, yet there were only two major peaks of DNA polymerase activities. Perhaps the complex was formed by the involvement of other matrix components. In fact, when the solubilized matrix was reacted with anti-T sera, several distinct host proteins (approx. M.W. 135K, 62K, 40K, 26K) in addition to the known 56K protein (nonviral T-antigen) were co-precipitated with SV40 T-antigen. The tight association of α-polymerase with these components, including T-antigen, may form a functional "domain" which can be liberated from the matrix. Whether any of these proteins are possible candidates responsible for the involvement of T-antigen and DNA polymerase association remains to be determined. The possibility that the matrix-bound polymerase represent a special form of α-polymerase will be further investigated after detailed fractionation of α-, β- and γ-polymerases from isolated nuclei. The direct involvement of the nuclear matrix in DNA replication will however require the reconstitution of the replication machinery using the nuclear matrix and its associated enzymatic activities to demonstrate the DNA synthesis *in vitro*.

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