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Stimulation of DNA Polymerase α by a Nuclear DNA/Protein Complex

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A nuclear DNA complex containing DNA polymerase and SV40 T-antigen was isolated from nuclei of SV40-transformed mouse fibroblasts. DNA polymerase could be separated from the complex. The remaining DNA/T-antigen-containing complex stimulated DNA polymerase α activity about 10-fold. The complex contained 4 major proteins with molecular weights of 46, 54, 76, and 94 kilodalton (KD). The stimulation activity was retained by protein A–Sepharose loaded with specific IgG from SV40-tumor bearer serum, or from antisera against the 94 KD and 76 KD components and was partially inhibited in the presence of these antisera. The stimulation activity was completely abolished by treatment of the complex with trypsin or DNase I.

Key words: DNA polymerase α, nuclear DNA/protein complex, SV40 T-antigen

The gene A product of simian virus 40 (SV40), a polypeptide with an apparent molecular weight of about 94,000 [1–5], has been shown to control the establishment and maintenance of cellular transformation. Transformation with SV40 ts A mutants is not induced when the cells were infected at the nonpermissive temperature [6–13] and is reverted to a degree of normality when cells transformed at the permissive temperature were propagated at the nonpermissive conditions. In addition to a number of virus-related functions, there is also good eivdence that gene A product may control DNA synthesis in transformed cells [6, 14–16] and that direct intracellular microinjection of a highly purified T-antigen preparation can stimulate host DNA synthesis [17]. The definite molecular mechanisms by which the SV40 gene A product initiates and maintains cellular transformation, as well as controls cellular DNA synthesis, either directly or indirectly, have not yet been associated.

The potential association of the SV40 gene A product with the control of cellular DNA synthesis stimulated studies to correlate the presence of SV40 T-antigen in SV40-transformed cells with a molecular function influencing DNA polymerase activity. The approach selected for this problem aimed at the isolation of nuclear DNA/protein com-

Abbreviations: TTP = thymidine-5′-triphosphate, dATP = deoxyadenosine-5′-triphosphate, dGTP = deoxyguanosine-5′-triphosphate, dCTP = deoxycytidine-5′-triphosphate, di-TTP = 2′-3′-dideoxythymidine-5′-triphosphate, PBS = phosphate buffered saline, DTT = dithiothreitol, SDS = sodium dodecyl sulfate, and KD = kilodalton.

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plexes that contain SV40 T-antigen as well as DNA polymerase. This report describes a series of experiments that characterize the interaction between SV40 T-antigen-containing complexes and DNA polymerase.

MATERIALS AND METHODS

Chemicals

(^{3}H)TTP (47 Ci/mmole) was purchased from Amersham-Buchler (Braunschweig, Germany); calf thymus DNA type I, DNase I, N-ethylmaleimide, TTP, dATP, dGTP, dCTP from Sigma (Munich, Germany); poly (A) $(dT)_{12}$ and poly(dA) poly(dT) from Boehringer (Mannheim, Germany); 2′-3′-dideoxythymidine-5′-triphosphate (di-TTP) from P. L. Biochemicals (Milwaukee, WI); and trypsin inhibitor (Trasylol) was kindly supplied by Bayer (Elberfeld, Germany).

Cells

STU-51 A/232B mouse cells, a subline derived from SV40-transformed embryonic fibroblasts of STU mice [18], were grown at 37°C in suspension cultures. The cell population, maintained in logarithmic growth by adequate dilution with fresh medium every 24 h, was used for the preparation of SV40 T-antigen-containing complexes. The cells were cultivated in Dulbecco's modified Eagle's essential medium supplemented with 10% inactivated fetal calf serum.

Preparation of SV40 T-Antigen and DNA Polymerase-Containing Complexes

The cells were washed 3 times in ice-cold phosphate buffered saline, pH 7.2 (PBS), and nuclei were prepared according to a slightly modified method described by Penman [19]. Packed cells (3.2×10^9) were resuspended in 10 volumes of 1:2 diluted reticulocyte buffer (RSB, composed of NaCl, 0.01 M; Tris/HCl, 0.1 M, pH 6.0; $MgCl_2$, 1.5 mM) and allowed to swell for 5 min at room temperature. Disruption was achieved in a glass Dounce homogenizer by 6 strokes with a tight-fitting pestle. The nuclei were sedimented by low-speed centrifugation at 4°C and 100g for 5 min. The nuclear pellet was washed twice with ice-cold RSB solution and kept frozen at −80°C until used. The samples of nuclei contained less than 5% of intact cells.

The thawed packed nuclei (5–10 ml) were diluted 1:1 with a stock buffer solution to give the final concentrations of 0.04 M Tris/HCl, pH 9.0 (adjusted at 4°C), 0.05 mM $CaCl_2$, 1 mM EDTA, and 10% glycerol without any detergents or protease inhibitors. The mixture was transferred to polyallomer tubes and exposed to nine 15 s cycles of sonication (70 W, Branson ultrasonic desintegrator) in ice water with 15 s intervals. The sonicate was adjusted to 0.15 M NaCl/1 mM DTT and then treated with RNase A (25 μg/ml sonicate) and stirred in an ice bath for 2 h. The resulting nuclear lysate was subjected to high-speed centrifugation at 40,000 rpm for 30 min in a Spinco 50 Ti rotor. The clarified supernatant was precipitated between 20% and 60% saturation with $(NH_4)_2SO_4$, pH 8.0, during 2 h at room temperature. The ammonium sulfate precipitates were redissolved in 5.0 ml of 0.04 M Tris/HCl buffer, pH 9.0, and dialysed for 3 h against 2 changes of 100 volumes of the same buffer.

The dialysate containing 100–150 mg of protein was adjusted to 0.04 M Tris/HCl, pH 9.0, containing 0.15 M NaCl, 1 mM EDTA, 1 mM DTT, and 10% (v/v) glycerol and was loaded onto an ultrogel AcA 22 (LKB) column (5 × 70 cm) equilibrated and eluted with the same buffer. Fractions of 4.0 ml were collected at a flow rate of about 40 ml/h. Com-

plement fixation-positive and DNA polymerase fractions were separately pooled and stored at −20°C until used. Normal rabbit serum dialysed against elution buffer was used for calibration of the column.

Chromatography of the DNA polymerase-stimulating fractions (from Fig. 1c) on double-stranded DNA cellulose columns [20] was carried out by stepwise elution using 0.15, 0.3, 0.6, 1.0, and 2.0 M KCl. Only calf thymus DNA was used for binding.

Determination of the protein content was performed by the method of Lowry et al [21].

Routine Assay for DNA Polymerase and Stimulation

Twenty-five microliters of assay buffer containing 0.1 M Tris/HCl, pH 8.0; 1 mM $MgCl_2$; 40 mM KCl; 1 mM DTT; 100 μM each of dATP, dCTP, and dGTP; 10 μM TTP; 0.7 μCi of (^{3}H)TTP (47 Ci/mmole); 25 μg bovine serum albumin; 5% glycerol; and 25 μg activated calf thymus DNA, prepared according to the method of Schlabach et al [22], were mixed with 10 μl of the fraction expected to contain DNA polymerase, 10 μl of column buffer 0.04 M Tris/HCl, pH 9.0, containing 0.15 M NaCl, 1 mM EDTA, 1 mM DTT and 10% glycerol, and 5 μl of a bovine serum albumin solution (10 mg/ml). In case of assaying stimulation activity 25 μl of assay buffer was mixed with 10 μl of the DNA polymerase fraction containing 100–130 ng protein, 10 μl of column fractions to be tested for stimulation activity, and 5 μl of bovine serum albumin solution as mentioned above. The mixtures were incubated at 37°C for 30 min. The reaction was terminated by addition of 50 μl 0.01 M Tris/HCl, pH 7.2, with carrier calf thymus DNA (2.5 mg/ml), followed by precipitation at 4°C with 10% trichloroacetic acid containing 10 mM sodium pyrophosphate. Glass fiber filters GF/C (Whatman) were used to collect radioactive insoluble precipitates.

Assays for DNA polymerase α, β, and γ were performed according to the outlines given by Edenberg et al [23].

Complement Fixation

Serum was obtained from Syrian hamsters bearing SV40-induced transplantable tumors. SV40-T-antigen was assayed by complement fixation [24]. The anti-SV40 T sera used had titers of at least 1:256 against nuclear homogenates of STU 51A/232B cells and less than 1:8 against nuclear homogenates of mouse embryonic cells.

Preparation of Rabbit Antisera Against Single Components of the T-Antigen Complex

A pooled T-antigen complex fraction (see Fig. 1d) containing 18 mg of protein was subjected to SDS-polyacrylamide slab gel electrophoresis. The 4 major bands with 94, 76, 54, and 46 KD (Fig. 3) were located, excised, minced, dialysed against PBS, and emulsified with complete Freund's adjuvant. The individual samples were injected into rabbits, with 2 injections being applied at an interval of 4 weeks. Antisera were obtained 7 days after the last injection.

Immune Adsorption of the T-Antigen Complex

Preparation of IgG fractions by gel permeation on Sephadex G-200 columns was performed with hamster and rabbit preimmune sera, sera of SV40-tumor-bearing hamsters, and the monospecific rabbit antisera against the individual components 94, 76, 54, and 46 KD of the T-antigen complex. Columns (0.3 or 0.6 ml) of protein-A-Sepharose CL-4B (Pharmacia, Freiburg, Germany) equilibrated with 0.02 M Tris/HCl, pH 8.8, containing 0.08 M KCl, 0.5 mM EDTA, 0.5 mM DTT, and 5% glycerol, were loaded with 7 mg of the

individual IgG fractions in 200 μl of the same buffer, followed by 200 μl of the corresponding sera, and then washed with 2 ml of column buffer. Thereafter, 200 μl of the T-antigen complex fraction containing 140 μg of protein were subjected to each column and eluted with column buffer.

The eluates were assessed for DNA polymerase stimulation according to the routine assay conditions, with incubation periods of 15 min but without addition of bovine serum albumin.

Immune Inhibition of DNA Polymerase Stimulation

Fifty microliter portions of the T-antigen complex fraction (containing 63 μg protein) were preincubated with 50 μl portions of the various antisera at 4°C up to 60 h. Ten-microliter portions of the preincubation mixture were assessed for stimulation of DNA polymerase activity using 30-min incubation periods.

RESULTS

Characterization of Nuclear DNA/T-Antigen Complexes

Nuclear DNA/T-antigen complexes were found in extracts obtained after stepwise disruption of nuclei by ultrasonication. The extract after short treatment by 4 sonication cycles (each 15 s; 50 W) contained DNA/T-antigen complexes that appeared to be smaller than 2×10^6 dalton when chromatographed on Biogel A 50 m columns. T-antigen-specific complement fixation (serum from tumor-bearing hamsters) and DNA polymerase activities were detected in about the same fractions. Chromatography of the same extract on ultrogel AcA-22 columns showed that under these conditions most of the DNA polymerase activity was separated from the peak of T-antigen-specific complement fixation, but a minor part of DNA polymerase still comigrated with the DNA/T-antigen complex, with an apparent complex size somewhat higher than 1×10^6 dalton (Fig. 1a).

More intensive sonication of nuclei for a longer time (9 cycles of 15 s; 70 W), followed by high-speed centrifugation to remove particulate material led to complete separation of two well-defined peaks of DNA polymerase activity from the DNA/T-antigen complex (Fig. 1b), ranging from fraction 140 to fraction 200, with a residual activity between fractions 200 and 230. When the high-speed supernatant was subjected to ammonium sulfate fractionation, before column chromatography, both activities (T-antigen and DNA polymerase) were precipitated together between 20% and 60% saturation. Chromatography of the dissolved precipitate on ultrogel AcA-22 columns exhibited about the same elution pattern (Fig. 1c), sometimes with a third DNA polymerase peak between fractions 200 and 230. When the pooled T-antigen-containing fractions were reconcentrated by ammonium sulfate precipitation (60% saturation) and rechromatographed on the same column, the DNA/T-antigen complex was found to be stable and eluted from the column at the same positions (Fig. 1d). Further criteria on complex stability are given under "Stimulation of DNA Polymerase by T-Antigen-Containing Complexes."

A more intensive sonication of the nuclei (8 cycles, 15 s; 90 W) resulted in a partial disintegration of the DNA/T-antigen complex, and part of the complement fixation-positive material was then eluted together with DNA polymerase, resulting in an apparent increase of DNA polymerase activity (not shown).

To characterize the protein composition of the DNA/T-antigen complex SDS-gel electrophoresis of the rechromatographed sample (Fig. 1d) was performed. It was found that in the complex several major proteins with molecular weights of 120,000, 94,000, 76,000,

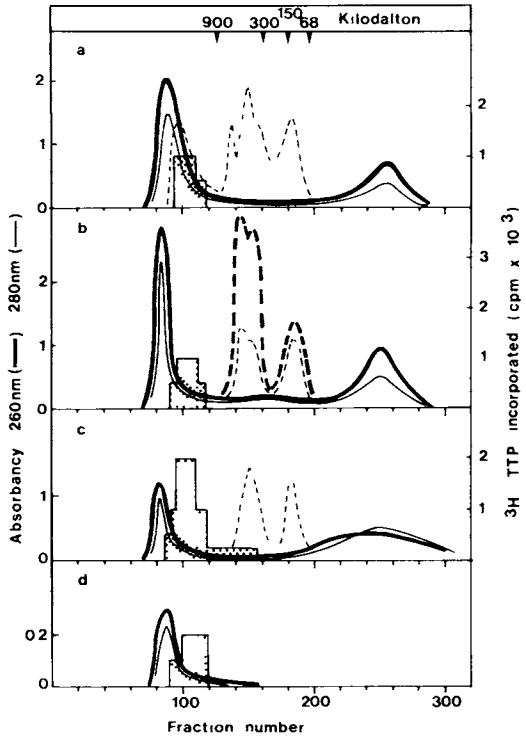

Fig. 1. Separation of the T-antigen-containing complex from DNA polymerase activites by ultrogel AcA-22 filtration after ultrasonication of the nuclei: a) Supernatant after sonication at 50W in 4 cycles of 15s and centrifugation at 3000g for 20 min using 5 ml of packed nuclei. b) The sonication was made at 70 W in 9 cycles of 15s. The sonicate was centrifuged in a Spinco Ti-50 rotor at 40,000 rpm for 30 min at 4°C, and the supernatant applied to the column. c) The supernatant of the sonicate from b was first subjected to $(NH_4)_2SO_4$ fractionation. Precipitates were sedimented by centrifugation in a Spinco rotor 30 at 15,000 rpm for 15 min at room temperature, dissolved and dialysed against elution buffer, and applied to the column. d) T-antigen-containing fractions of c were pooled, precipitated by ammonium sulfate at 60% saturation, sedimented by centrifugation, redissolved in 3 ml 0.04 M Tris/HCl buffer, pH 8.0, dialysed against the same buffer, and applied to the column. Dotted area, SV40-T-antigen-specific complement fixation; dashed line, DNA polymerase activity given in cpm (^{3}H)TTP incorporated; heavy dashed line, stimulation of DNA polymerase activity by the DNA/T-antigen complex given in cpm (^{3}H)TTP incorporated.

54,000, and 46,000 (accuracy, ±5%) were present (Fig. 2). In some preparations, the 120,000 dalton component was missing. These proteins were generally also found in SV40 T-antigen-specific immunoprecipitates from homogenates of SV40-transformed cells [25–29, 39, 40].

Stimulation of DNA Polymerase Activity by T-Antigen-Containing Complexes

Complete separation of DNA polymerase activity from T-antigen-specific complement-fixation activity (DNA/T-antigen complexes) showed an apparent loss of enzymatic activity when compared to the activity present in the original sample. This observation suggested that the DNA/T-antigen complex may be, or at least may contain, a stimulatory factor for DNA polymerase activity. To prove this assumption, the activity of DNA poly-

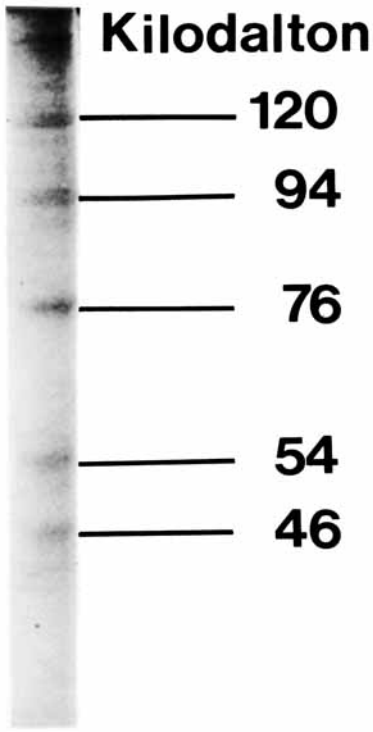

Fig. 2. SDS-Polyacrylamide gel electrophoresis of the rechromatographed DNA/T-antigen complex from nuclei of SV40-transformed mouse cells described in Figure 2d. Protein standards were β-galactosidase (115,000), phosphorylase a (96,000), bovine serum albumin (68,000), horse γ-globulin heavy chain (53,000) and light chain (22,000), and tobacco mosaic virus (17,500). Accuracy of molecular weights indicated: ±5%.

merase activity was assayed with and without the addition of the T-antigen-containing fractions, as well as with other column fractions. To make sure that the fractions to be tested were free of endogenous DNA polymerase activity, they were preincubated at 50°C for 5 min, which was found to completely abolish enzyme activity but to retain T-antigen-specific antibody-binding capacity [30]. DNA polymerase activity eluted from the AcA-22 column (Fig. 1b) was represented by two main peaks of activity under our assay conditions. Only one of these was stimulated by more than 100% when the T-antigen-containing pool fraction from the same column was added (Fig. 1b). All the other column fractions did not show the same significant stimulation effect. When the sample was treated with DNase I at 37°C for 20 min before AcA-22 column chromatography, neither fraction eluted from the column showed significant DNA polymerase stimulation, and T-antigen activity was essentially distributed over the entire elution pattern.

After chromatography of the DNA polymerase-stimulating complex (see Fig. 1d) on double-stranded DNA cellulose, stimulation activity and T-antigen-specific complement fixation were eluted together in one peak, with 0.15 M KCl exhibiting a coinciding distribution of activities.

Kinetic studies showed that DNA polymerase activity, as well as stimulation activity of the T-antigen-containing complex, persists for more than 6 h. As shown in Figure 3a, there is apparently no detectable lag period for stimulation, and DNA synthesis is stimulated

by the complex also 1 hour after the initiation of the reaction. The rate of incorporation was dependent on the concentration of the T-antigen-containing complex present in the reaction (Fig. 3b). For the given constant amount of DNA-polymerase, the saturation plateau was achieved at about 700–900 ng of protein of DNA/T-antigen complex, indicating strongly that the phenomenon of stimulation is characterized by defined molecular ratios of T-antigen complex and DNA polymerase.

Addition of ATP to support endogenous phosphorylation did not affect the stimulation capacity of the DNA/T-antigen complex; thus, one has to assume that the DNA/T-antigen complex is either fully phosphorylated or that phosphorylation is not essential for DNA polymerase stimulation.

Biochemical Criteria of the Stimulation Effect

In order to find out to which of the three major DNA polymerase classes the stimulated DNA polymerase activity (Fig. 1b, first DNA polymerase peak) belongs, primer-template specificity and optimal reaction conditions were characterized. Control experiments had shown that the pool fractions of DNA polymerase and DNA/T-antigen complex did not contain an endogenous template. The DNA polymerase activity was completely abolished in the presence of 0.5 mM N-ethylmaleimide, indicating that DNA polymerase β is not present. Using different primer-template and salt conditions able to differentiate between DNA polymerase α and γ [23], highest DNA polymerase activity was observed with activated calf thymus DNA compared with native (dsDNA) or single-stranded calf thymus DNA (ssDNA) or the synthetic polymers poly(A) $(dT)_{12}$ and poly(dA) poly(dT) (Table I). These results showed that the main enzymatic activity in this peak was DNA polymerase

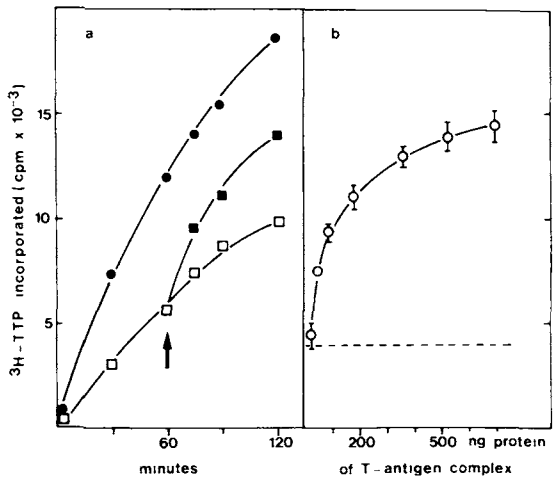

Fig. 3. Stimulation of DNA polymerase activity from nuclei of SV40-transformed mouse cells by the DNA/T-antigen complex obtained from the same cells as described in Figure 2. The DNA polymerase samples tested corresponded to first DNA polymerase peak in Figure 2b. a) Kinetics of incorporation of (^{3}H)TTP into DNA in the presence (•—•) and absence (□—□) of DNA/T-antigen complex, (▪—▪) addition of T-antigen complex to the reaction mixture 1 h after the start of experiment using standard assay conditions described under Methods. b) Stimulation of DNA polymerase activity by various concentrations of a concentrated DNA/T-antigen complex pool fraction during a reaction time of 30 min. Individual samples contained 1 μg protein of DNA polymerase active fractions, 5 μl bovine serum albumin (10 mg/ml), various amounts of concentrated and preincubated (5 min at 50°C) DNA/T-antigen complex, and standard assay buffer in a final volume of 50 μl.

TABLE I. Primer-Template and Buffer Specificity of DNA Polymerase Activity Stimulated by the DNA/T-Antigen (Tag) Complex*

	Conditions of (^{3}H)TTP incorporation (cpm) optimal for					
	DNA polymerase α [23]		DNA polymerase α + 100 mM KCl [31]		DNA polymerase γ [23]	
Primer template	− (DNA/Tag) complex	+ (DNA/Tag) complex	− (DNA/Tag) complex	+ (DNA/Tag) complex	− (DNA/Tag) complex	+ (DNA/Tag) complex
Activated DNA	5,334	56,990	10,950	29,528	7,522	11,408
ss DNA	546	524	378	305	632	664
ds DNA	344	196	198	212	252	828
Poly(A) $(dT)_{12}$	796	376	690	794	834	930
Poly(dA) poly(dT)	1,218	696	1,834	504	804	1,218
−	nt[a]	656	nt	nt	nt	nt

*Nuclei of SV40-transformed mouse fibroblasts were isolated from logarithmically growing cells followed by ultrasonication of the nuclei and separation of DNA polymerase from the DNA/T-antigen complex by gel filtration on an ultrogel AcA-22 column as described in Figure 2. Primer-template specificity of the DNA polymerase fraction was determined under specific assay conditions [23, 31] in the presence and absence of DNA/T-antigen complex. The DNA polymerase sample tested was the first DNA polymerase peak in Figure 2b.

[a]nt = not tested.

α. However, DNA polymerase activity was increased when the assay buffer was supplemented with KCl. Maximum activity was found at 100 mM KCl. These findings correlate with the results obtained with a modified nuclear DNA polymerase α in a case of acute lymphoblastic leukemia [31]. Furthermore, the DNA polymerase activity was only partially affected by di-TTP at ratios diTTP/TTP up to 20:1, showing a decrease of less than 30% at all conditions. The relative inhibition of DNA polymerase activity was not altered in the presence of the DNA/T-antigen complex. This insensitivity of our DNA polymerase activity to di-deoxy-thymidine triphosphate further indicates that the polymerase activity stimulated by the DNA/T-antigen complex is predominantly represented by DNA polymerase α, as known from other investigations [23].

The stimulation of DNA polymerase activity by the DNA/T-antigen complex under the reaction conditions of DNA-polymerase α [23] was observed only with activated calf thymus DNA, and maximal stimulation was observed in the absence of KCl (Table I).

For the characterization of the structural properties of the DNA/T-antigen complex responsible for stimulation of DNA polymerase, the complex was pretreated with trypsin or DNase I. Tryptic digestion (15 min at 37°C) was terminated by addition of trypsin inhibitor to avoid destruction of DNA polymerase after mixing, and digestion of the complex with DNase I (10 min at 37°C) was terminated by heating (10 min at 80°C) to avoid degradation of the newly synthesized DNA. In Table II, the experiments of stimulation of DNA polymerase activity by the pretreated DNA/T-antigen complex are listed together with the corresponding controls. The ability to stimulate DNA polymerase α was abolished when the DNA/T-antigen complex was pretreated either with trypsin or DNase I. Only (^{3}H)TTP incorporation corresponding to the DNA polymerase control was observed, provided that enzymatic degradations were stopped before mixing. The stimulation activ-

TABLE II. Stimulation of DNA Polymerase Activity After Treatment of the DNA/T-Antigen (Tag) Complex With Trypsin or DNase I*

		Pretreatment of the DNA/T-antigen complex with:				
DNA polymerase	DNA/Tag complex	Trypsin	Trypsin inhibitor	DNase	Heating 10 min, 80°C	cpm (^{3}H)TTP incorporated
+	–	–	–	–	–	1,333
+	+	–	–	–	–	7,488
+	+	+	–	–	–	142
+	+	+	+	–	–	1,496
+	+	–	–	+	–	195
+	+	–	–	+	+	1,608
+	+	–	–	–	+	7,011
–	+	+	–	–	–	291
–	+	+	+	–	–	204
–	+	–	–	+	–	257
–	+	–	–	+	+	154
–	+	–	–	–	–	225

*Extraction of nuclei and separation of DNA polymerase from the DNA/T-antigen complex was carried out as described in Figure 2. For the assessment of DNA polymerase α, activity-specific assay conditions [23] were used. The pretreatment of the DNA/Tag complex with trypsin (15 min at 37°C) was terminated by addition of trypsin inhibitor, and treatment with DNase I (10 min at 37°C) was terminated by heating to 80°C for 10 min when indicated.

ity of the DNA/T-antigen complex was not significantly altered by heating to 80°C for 10 min. Furthermore, it is noteworthy that although the stimulation activity of the complex was destroyed by DNase treatment, the T-antigen-specific complement fixation remained entirely intact.

Similar experiments designed to characterize the DNA-polymerase class of the second peak of DNA-polymerase activity (Fig. 1b), which is not stimulated by the DNA/T-antigen complex, showed that in this peak also the enzyme activity is typical for DNA-polymerase α. The frequently obtained third DNA polymerase peak is a mixture of polymerases.

Effect of Specific Antibodies on Stimulation Activity

The T-antigen complex fraction (Fig. 1d) might still represent a crude complex associated with components of low structural affinity. Therefore, experiments were performed to selectively retain components of the T-antigen complex fraction by specific antibodies bound to protein-A-Sepharose. Protein-A-Sepharose columns were prepared with IgG fractions of monospecific antisera against the 46, 54, 76, or 94 KD component, as well as with IgG from SV40 tumor-bearer serum and preimmune hamster and rabbit serum. After immune adsorption of the T-antigen complex fraction, DNA polymerase stimulation activity was assessed in the eluate. In Table III, the results are listed for a typical experiment. The stimulation activity was never decreased when the T-antigen complex fraction was tested after adsorption to IgG specific for the components 54 KD and 46 KD. The decrease of DNA polymerase stimulation ranged between 0 and 43% after adsorption to anti-76 KD IgG, 38% and 63% after adsorption to anti-94 KD IgG, and between 21% and 61% after adsorption to IgG of tumor-bearer serum, dependent on various specific antisera used for IgG preparation. Immune adsorption of the T-antigen complex to these specific IgG columns yielded stronger decreases of DNA polymerase stimulation when the larger protein-A-Sepharose columns were used.

To find out whether specific antibodies can directly inhibit stimulation of DNA polymerase by the T-antigen complex, the T-antigen complex fraction was preincubated with the specific antisera and then assessed for DNA stimulation without removing the immune complexes. The stimulation activity was not influenced by antisera against the 46 KD and 54 KD components, but was inhibited up to 30% when antiserum against the

TABLE III. Effects of Immune Adsorption of the DNA/T-Antigen (Tag) Complex Fraction by IgG-Protein-A-Sepharose Columns on Stimulation of DNA Polymerase*

DNA polymerase	DNA/Tag complex	Adsorbing IgG fraction	Incorporation, cpm (^{3}H)TTP	Decrease of stimulation (%)
+	–	–	2,311	–
+	+	Preimmune serum	7,353	0
+	+	Tumor-bearer serum	4,630	54
+	+	Anti-94 KD	4,176	63
+	+	Anti-76 KD	6,294	21
+	+	Anti-54 KD	7,728	0
+	+	Anti-46 KD	7,518	0

*The DNA/T-antigen-containing complex (from Figure 2d) was chromatographed on freshly prepared 0.3 ml protein-A-Sepharose columns loaded with the IgG fraction of the antisera given. The eluates were assessed for DNA polymerase stimulation according to the routine assay conditions.

76 KD component was used and up to 56% after preincubation with antiserum against the 94 KD component. In the presence of serum from tumor-bearing hamsters, inhibition of the stimulation of DNA polymerase by the T-antigen complex did not exceed 34%.

DISCUSSION

Several reports indicated that infection with papovaviruses influences the synthesis of cellular DNA in infected cells. However, the molecular mechanisms by which this occurs are not understood. Any effort in elucidating this process will be of great help in understanding the general mechanisms by which DNA synthesis is controlled. SV40-infected or polyoma virus-infected mouse cells show an approximately 10-fold increase of nuclear DNA polymerase α activity, compared to a 2-fold increase in the cytoplasm [32, 33]. This increase in polymerase activity was also observed when the infection was carried out in the presence of 5-fluorodeoxyuridine, known to prevent viral and cellular DNA replication [34], thus indicating that no new viral polymerase activity was induced by SV40. In the polyoma virus system, elevation in host cell DNA polymerase activity in the infected cell requires preceding synthesis of viral-specific T-antigen [35].

In this paper, we have described the existence of a DNA/T-antigen complex able to stimulate DNA polymerase α approximately 10-fold. The existence of this complex in SV40-transformed cells could explain the observation reported on the effect of SV40 or polyoma infections on cellular DNA replication.

The cells used in this work are a permanent cell line transformed by SV40, and it can be speculated that the T-antigen present in these cells might substitute for, and/or compete with, a cellular protein, that is normally active in dividing cells and inactive in nondividing cells, thus controlling DNA synthesis as was proposed for virus-specific pleiotropic effectors in transformed cells [36].

The isolation of a DNA polymerase-α-stimulating nuclear complex might be an important step in the characterization of the control of DNA synthesis in these cells. T-antigen appears to be an essential part of this nuclear complex, as was shown by our immune adsorption experiments. The IgG fraction of the monospecific antiserum against the 94 KD component retained the stimulating complex as well as the IgG fraction from SV40-tumor-bearing hamsters when bound to protein-A-Sepharose columns. Since tumor-bearer sera did not contain antibodies reacting with nuclear components of embryonic cells, the inhibitory effect of antisera against the 94 KD protein is consistent with the conclusion that the inhibitory action is directed against T-antigen. The presence of the 76 KD component in the stimulating complex is more difficult to interpret. It might be identical with a protein that exhibits T-antigen specificity, but its formation appears to be dependent on the degree of proteolysis during the extraction and handling of samples [41–46]. It might also represent a distinct state of T-antigen processing. The stimulating complex was not retained in columns with IgG from monospecific antisera against the 54 KD component. This is somewhat surprising since a specific complex of SV40 T-antigen (94KD) with a host protein with 53 KD was described recently [47, 48]. However, our monospecific antisera were obtained with denatured substances, and the specific antibodies might possibly not cross-react with the specific conformation of this protein bound in the stimulating complex. The same argument might apply to the 46 KD component.

Several investigators have detected protein components in SV40 T-antigen-specific immunoprecipitates [25–29, 39, 40] analogous to the protein components found in our DNA/T-antigen complex. These findings can be interpreted by the possibility that SV40 T-antigen-specific antibodies precipitate the entire DNA/T-antigen complex.

Our results of the direct inhibition of stimulation of DNA polymerase by specific antisera showed that the anti-94 KD serum is a more potent inhibitor than sera of tumor-bearing hamsters or the anti-76 KD serum. These differences can be explained by the fact that the specific antibodies of tumor-bearer sera (and possibly also of anti-76 KD sera) are preferentially directed against different sites of T-antigen [49], which are not fully accessible in the stimulating complex. The findings do not yet allow a discrimination of two potential functions of T-antigen in the stimulating complex: Is T-antigen directly involved in stimulation, or is it only influencing the regulation of DNA polymerase stimulation? The partial inhibition by anti-94 KD sera might also derive from sterical hindrance of DNA polymerase stimulation. Anti-54 KD serum and anti-46 KD serum did not inhibit DNA polymerase stimulation by the T-antigen complex, as could be expected from the immune adsorption experiments.

The findings that the stimulation capacity of the nuclear DNA/SV40 T-antigen complex was sensitive to degradation with trypsin and DNase indicates the involvement of the entire structure of the complex, possibly with a distinct spatial arrangement of the intrinsic components that is stable enough to withstand heating at 80°C. When isolated under mild conditions, this complex appears to be associated with DNA polymerase α, giving the enzyme a higher activity than in its isolated form. This may indicate that this associated structure represents the DNA-replicating complex in the nucleus of the transformed cell.

The molecular criteria of stimulation of DNA polymerase α by the DNA/SV40 T-antigen complex appear to be different from those of stimulation of DNA polymerase by an adenovirus type 12 tumor antigen-containing fraction [37]. In this case, the authors reported a more significant stimulation with native calf thymus DNA as a primer-template than with the activated DNA sample. The complex that we describe has no stimulatory effect on either native or denatured DNA. This also differentiates it from the basic protein described in HeLa cells [38], which stimulates DNA polymerase α activity only in the presence of single-stranded DNA. The existence of several factors, described in the literature [37, 38], able to stimulate DNA polymerase activity, together with the DNA/T-antigen complex we describe here, indicates that one of the possible mechanisms of control of DNA synthesis might be the regulation of the DNA polymerase activity involved.

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