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DNA Polymerase Activities in Growing Cells Infected with Simian Virus 40

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Growing CV_1 cells were infected with simian virus 40 (SV40), and the levels of DNA polymerases-α, -β, and -γ were analyzed in the cytoplasm, nuclear Triton wash, and nucleus. In the cytoplasmic fraction, the amount of α-, β-, or γ-polymerase remained unaltered after SV40 infection. The activity of DNA polymerase-α increased five- to sixfold in the nuclear Triton wash and threefold in the nuclei and then remained enhanced only inside the nuclei. That of DNA polymerases-β and -γ increased mostly in the nuclei after infection. These results suggest that DNA polymerase-α could be the major enzyme involved in SV40 DNA replication.

Productive infection of resting confluent green monkey kidney cells (9, 10) by simian virus 40 (SV40) results not only in SV40 DNA synthesis but also in the induction of cellular DNA synthesis.

Earlier studies have shown that a variety of cellular enzymes involved in the synthesis of DNA were concomitantly induced by SV40 or polyoma infection of resting cells (11, 12). Three DNA polymerase activities have been identified since then in mammalian cells (see references 3 and 29 for a review), but which of these enzymes is involved in DNA replication or repair is not clearly known. DNA polymerase-α is predominantly present in the cytoplasm; DNA polymerase-β is present in the nucleus, and DNA polymerase-γ is localized in both the cytoplasm and nucleus. These three enzymes can be distinguished by their capacities to copy different synthetic templates, their molecular weights and sedimentation coefficients, and their in vitro sensitivities to *N*-ethylmaleimide. Circumstantial evidence for the involvement of DNA polymerase-α in DNA replication has been found in various experimental systems, such as synchronized mouse L cells or HeLa cells (6, 26) or regenerating rat liver (2, 3, 7). DNA polymerase-γ activity also seems to increase in cells entering the S phase (26), but its origin and role in replication are unknown (A. Weissbach, personal communication).

It would be interesting to know which of these enzymes is induced after infection with SV40 or polyoma virus. Recently, Wintersberger and Wintersberger have observed that infection of resting mouse kidney cells by polyoma virus results in an induction of DNA polymerase-α in the cytoplasm and nucleus which is linked to active DNA synthesis (31).

In contrast to what is observed with resting cells, polyoma virus or SV40 infection does not induce cellular DNA synthesis in growing cell cultures (18, 24, 25). In a previous work, one of us showed that infection of resting CV_1 cells resulted in slow and asynchronous viral multiplication, whereas infection of the same type of cells in their growing phase resulted in short viral cycles (about 50 h) without stimulation of cell DNA synthesis (18, 22). Since in such a system DNA polymerase activities required for the replication of cellular DNA are already present at the time of infection, we attempted to determine whether SV40 infection modified the level and/or intracellular location of any of the various DNA polymerase activities already present in the growing cells. This was done to possibly detect the DNA polymerase(s) effectively involved in SV40 DNA replication.

MATERIALS AND METHODS

Poly(dC) and poly(dT) homopolymers were obtained from P. L. Biochemicals; oligo$(dG)_{12-18}$ was from Collaborative Research Inc.; poly(A) was from Miles Laboratories, Inc.; and poly(A)·$(dT)_{12-18}$ (base ratio, 1:1) was from Boehringer, Mannheim Corp. Synthetic duplexes were annealed by heating at 80°C followed by slow cooling. Poly(A·dT) was annealed at a ratio of 1:2, and poly(dC)·$(dG)_{12-18}$ was annealed at a ratio of 1:4 (in mononucleotide concentrations).

Activated calf thymus DNA was prepared by the method of Loeb (15) until 20% of the DNA had been made acid soluble by pancreatic DNase I.

Cells and infection. Cells from the cloned CV_1 subline previously described (18) were grown in minimum Eagle medium supplemented with 10%

tryptose phosphate and 10% calf serum (MCV_1). The cell cultures were repeatedly found mycoplasma-free, as judged by either microscope examination after staining with Giemsa or electron microscopy.

For the experiments reported here, the cells were seeded in 10-cm plastic petri dishes at 8×10^5 cells per dish in MCV_1. The culture medium was changed after 48 h, and the cells were infected on day 3 with 0.5 ml of the large-plaque strain of SV40. Input multiplicity was 20 PFU/cell. After 90 min of adsorption at 37°C, the cell monolayers were overlaid with 10 ml of MCV_1 and incubated at 37°C under 5% CO_2. Mock infection of cells was done similarly, except that 0.5 ml of MCV_1 was used in place of the virus suspension.

Cells from 15 petri dishes (8×10^7 to 1×10^8 cells) were used for each cell fractionation. The cell monolayers were washed with cold phosphate-buffered saline solution and scraped from their support in the presence of phosphate-buffered saline solution containing 0.2 mM EDTA. Cell number was determined with a hemacytometer. The cells were washed with 50 mM Tris-hydrochloride (pH 7.5)–25 mM KCl–3 mM $MgCl_2$–2 mM $CaCl_2$ (TKCM) containing 0.25 M sucrose, suspended in the same buffer (25×10^6 cells/ml), and homogenized with 15 strokes in a Potter glass homogenizer tightly fitted with a Teflon pestle. Cell breakage, checked with the use of a microscope, was more than 95% complete without visible damage to the nuclei. The homogenate was centrifuged at $650 \times g$ for 10 min, and the supernatant fraction (cytoplasmic extract) was clarified by centrifugation for 60 min at 45,000 rpm in a 50Ti Spinco rotor. The nuclear pellet from the low-speed centrifugation was washed twice with 10 ml of 0.25 M TKCM and then layered over 2 ml of TKCM containing 0.6 M sucrose, and the nuclei were sedimented through this cushion ($2,500 \times g$, 15 min). The nuclear pellet was resuspended in 0.25 M TKCM containing 0.5% Triton X-100 to remove nuclear membranes (1). The suspension was allowed to stand for 15 min at 4°C, after which the nuclei were collected by centrifugation at $650 \times g$ for 10 min, while the supernatant (Triton extract) was further clarified by centrifugation for 60 min at 45,000 rpm in a 50Ti Spinco rotor. The nuclear pellet was resuspended in 0.25 M TKM (0.25 M TKCM without $CaCl_2$ and with 5 mM $MgCl_2$), layered again over a cushion of TKM containing 0.6 M sucrose, and sedimented for 15 min at $2,500 \times g$. The nuclei obtained were unaltered and free from nuclear membranes as judged by examination under an electron microscope.

Nuclear extracts were prepared by incubating the purified nuclei in 0.6 ml of 0.2 M potassium phosphate buffer (pH 7.5)–1 M KCl–2 mM EDTA–2 mM 2-mercaptoethanol for 2 h at 4°C with occasional mixing. The extract was clarified by centrifugation for 45 min at 45,000 rpm in the 50Ti Spinco rotor and then brought to 1 mg of bovine serum albumin (BSA) per ml. Cytoplasmic, nuclear, and Triton extracts were dialyzed against 40 mM Tris-hydrochloride (pH 8.0)–0.1 M KCl–1 mM dithiothreitol (DTT) before further analysis.

Sucrose gradient analysis. A 0.4-ml fraction of each of the dialyzed extracts was loaded onto a 5 to 20% sucrose gradient in 40 mM Tris-hydrochloride (pH 8.0)–0.2 M KCl–1 mM DTT, which had been layered on top of a 0.3-ml cushion of 60% sucrose in the same buffer. Centrifugation was performed for 15.5 h at 4°C in an SW50.1 Spinco rotor. The gradients were fractionated from the bottom into 28 equal fractions collected into tubes containing 90 μg of BSA. Equal portions of each of the fractions (usually 10 μl) were assayed for DNA polymerase activity as described below.

DNA polymerase assay. DNA polymerase activity was measured with different templates to distinguish α-, β-, and γ-polymerase activities (3, 29). Activated calf thymus DNA and poly(dC)·$(dG)_{12-18}$ were used as templates for DNA polymerases-α and -β, poly(A)·$(dT)_{12-18}$ was used for DNA polymerase-γ, and poly(dT·A) was used for DNA polymerase-α.

All incubations were carried out in a final volume of 50 μl. Incorporation of labeled nucleotide into acid-insoluble material was tested in initial velocity conditions under the conditions described below.

The assay using activated calf thymus DNA (400 μg/ml) was at 37°C in 50 mM Tris-hydrochloride (pH 8.0), 8 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 10% glycerol, 600 μg of BSA per ml, and 0.1 mM each dATP, dGTP, dCTP, and [^{3}H]dTTP (400 cpm/pmol).

The assay for DNA polymerase-α, using poly(dC)·$(dG)_{12-18}$ as a template (80 μM), was at 37°C in 50 mM Tris-hydrochloride (pH 8.0), 1 mM $MgCl_2$, 40 mM KCl, 5 mM 2-mercaptoethanol, 10% glycerol, 600 μg of BSA per ml, and 0.1 mM [^{3}H]-dGTP (180 cpm/pmol). That for polymerase-β, using 80 μM poly(dC)·$(dG)_{12-18}$, was in 50 mM Ammediol buffer (pH 8.8), 0.1 mM $MnCl_2$, 90 mM KCl, 0.2 mM DTT, 2 mM *N*-ethylmaleimide, 10% glycerol, 600 μg of BSA per ml, and 0.1 mM [^{3}H]dGTP (200 cpm/pmol). The amount of *N*-ethylmaleimide used was calculated to yield an inhibition over 98% of the DNA polymerase-α activity, without inhibition of DNA polymerase-β.

RNA-primed DNA synthesis catalyzed by DNA polymerase-α was assayed, using 50 μM poly(dT·A) in 50 mM Tris-hydrochloride (pH 7.6), 0.1 mM $MnCl_2$, 50 mM KCl, 2.5 mM DTT, 10% glycerol, 600 μg of BSA per ml and 0.1 mM [^{3}H]dATP (195 cpm/pmol).

DNA polymerase-γ activity was assayed with 80 μM poly(A)·$(dT)_{12-18}$ in 50 mM Tris-hydrochloride (pH 7.5), 0.5 mM $MnCl_2$, 140 mM KCl, 2.5 mM DTT, 10% glycerol, 600 μg of BSA per ml, and 0.05 mM [^{3}H]dTTP (850 cpm/pmol). Incubation was at 29°C, for at 37°C the activity was only 39% of that obtained at 29°C, probably due to the low melting point (28 to 30°C) of this template-primer hybrid. RNA-dependent DNA synthesis in this system is also detected with DNA polymerase-β (5).

After 30 or 60 min of incubation, 45 μl of the reaction mixture was transferred to a Whatman GF/C glass-fiber filter disk and processed for acid-insoluble material as previously described (8), and the radioactivity was determined by liquid scintillation counting.

The results were expressed as DNA polymerase units per 10^8 cells. One unit was defined as the amount of DNA polymerase activity required to con-

vert 1 nmol of total nucleotide into acid-insoluble material per h.

The use of poly(dC)·$(dG)_{12-18}$ as template allowed the detection of α- and β-polymerases under the better conditions, and thus it was chosen for the DNA polymerase assays after sucrose gradient fractionation.

Miscellaneous. DNA contents were determined colorimetrically by means of the diphenylamine test (4). Determination of T antigen was done by the micro-complement-fixation method of Wasserman and Levine (28), using sera from hamsters bearing SV40-induced tumors.

Purified regenerating rat liver DNA polymerase-α (6,300 U/mg) was prepared as previously described (8, 20). Purified regenerating rat liver DNA polymerase-β (3,000 U/mg) was a gift from J. M. Rossignol (Unité d'Enzymologie).

RESULTS

DNA polymerase activities in cell fractions. DNA polymerase activities were determined in nuclear, cytoplasmic, and nuclear membrane extracts, prepared as described above. Various methods used for the fractionation of cells were reviewed in preliminary experiments. The technique used for the preparation of nuclei was chosen because it gave a maximum and reproducible yield of nuclei of great purity, even with SV40-infected cells, without the decondensation of chromatin observed by the use of hypotonic mediums, as judged from electron microscopy examinations (data not shown). Nuclear membrane extracts refer to the 0.5% Triton washes of purified nuclei. This treatment removes both the inner and outer membranes of the nuclear envelope (1) by solubilizing over 95% of the nuclear phospholipid as well as the major polypeptides of the nuclear membranes, without release of histones or proteins from the nuclear pore complex. However, the nuclear Triton wash might contain proteins migrating between the cytoplasmic and nuclear compartments and, in fact, a DNA polymerase activity that is enhanced early in regenerating rat liver has been found in this extract (7). For convenience, we have called this fraction "nuclear membrane extract" without prejudice to the exact origin of the proteins contained. It was particularly interesting to analyze the DNA polymerase contents of these extracts in SV40-infected cells since it has been suggested that replicating SV40 DNA was associated with the host nuclear membrane (14).

Enzyme levels were determined in three sets of cultures: (i) mock-infected growing cells at 15 h after mock infection; (ii) infected growing cells at 15 h after infection; and (iii) infected growing cells at 39 h after infection. Three principal kinds of template were used for the assay of DNA polymerases. Activated calf thymus DNA was used to determine the total DNA-dependent DNA polymerase activity. Poly(dT·A) was used in the presence of [^{3}H]dATP to monitor the RNA-primed DNA synthesis that is specifically catalyzed by DNA polymerase-α (8, 27). This template was used since, as in polyoma DNA synthesis, RNA primers are probably involved in SV40 DNA replication (16). Poly(A)·$(dT)_{12-18}$ was used for the detection of DNA polymerase-γ, based on the capacity of this enzyme to generate RNA-dependent DNA synthesis (13, 26). However, in this assay, the activity detected in nonfractionated extracts is the sum of the activities of DNA polymerases-γ and -β since DNA polymerase-β can also use an RNA template for DNA synthesis (5).

Table 1 shows that infection of growing CV_1 cells with SV40 was not followed by any significant alteration of enzyme levels in the cytoplasmic fraction. On the contrary, infection resulted in a two- to threefold increase in both DNA- and RNA-dependent DNA polymerase activities in the nuclear fraction of the cell. This increase was stable between 15 and 39 h

TABLE 1. *DNA polymerase activities in cell extracts of SV40-infected cells*[a]

Cells	Template used	DNA polymerase activity (U/10^8 cells) in:		
		Cytoplasmic extract	Nuclear membrane extract	Nuclear extract
Mock-infected	Activated DNA	1,498	21	31
	Poly(dT·A)	208	3.4	1.8
	Poly(A)·$(dT)_{12-18}$	2.2	1.2	1.0
Infected at 15 h post-infection	Activated DNA	1,512	123	83
	Poly(dT·A)	218	25.6	4.4
	Poly(A)·$(dT)_{12-18}$	1.6	1.6	2.4
Infected at 39 h post-infection	Activated DNA	1,400	28	90
	Poly(dT·A)	192	3.8	5.0
	Poly(A)·$(dT)_{12-18}$	3.2	1.8	2.4

[a] DNA polymerase activities were measured as indicated in the text. Activated DNA was used for the total DNA-dependent DNA synthesis; poly(dT·A) was used for DNA-dependent DNA synthesis initiated by RNA, and poly(A)·$(dT)_{12-18}$ was used for the RNA-dependent DNA synthesis.

postinfection, in spite of the progression of the viral cycle (reference 18 and as checked by the accumulation of T antigen in the infected cells). In the nuclear Triton wash a pronounced enhancement of DNA-dependent activity was detected early in infection. Thus, at 15 h postinfection, DNA polymerase activity measured with activated calf thymus DNA was six times as high as in the control uninfected cells. RNA-primed DNA-dependent activity was also increased about seven times. Later in infection, however, both these activities decreased to approximately the control level. No significant alteration of DNA-primed RNA-dependent activities was found at 15 or 39 h postinfection.

These results suggest that SV40 infection of growing cells results in the first stage (15 h postinfection) in increased DNA-dependent DNA polymerase activity in both the nuclear membrane extract and nuclei. This enhancement appears to be transient in the nuclear membrane extract, but stable in the nuclei. The fact that such an increase was also detected with poly(dT·A) as a template suggests that it was an increase in the activity of DNA polymerase-α, since that template can only be used by DNA polymerase-α (8, 27; our unpublished data). RNA-dependent activity was also slightly enhanced in the host nuclei after infection, but this result should be interpreted with caution in view of the very limited amount of enzymatic activity in the cell fraction tested.

Characterization of DNA polymerase activities from SV40-infected cells. To ascertain these results further, the same cell extracts were fractionated by sucrose gradients, and the activity of each of the three different DNA polymerases was determined in the cytoplasmic, nuclear, and nuclear membrane fractions. The enzymes were identified according to their positions in the gradients, their template specificities, their salt dependence, and their inhibition by *N*-ethylmaleimide. All of these characteristic properties permitting the distinction of the α-, β-, or γ-enzyme from CV_1 cells have been studied in preliminary experiments and have been found to agree with the results previously obtained (3, 29).

Figure 1 shows the sedimentation profile of the DNA polymerase activities, using poly(dC)·$(dG)_{12-18}$ as a template in the presence or absence of 2 mM *N*-ethylmaleimide as a specific inhibitor of DNA polymerase-α.

In the cytoplasmic extract from mock-infected growing cells (Fig. 1, C1), the DNA dependent activity observed was due to DNA polymerase-α since it sedimented at 8.2*S* and was sensitive to *N*-ethylmaleimide. It should be noted that the low amount of DNA polymerase-β activity (0.6 U/10^8 cells) that was detected in the cytoplasmic extracts after sucrose gradient fractionation cannot be observed in Fig. 1, owing to the scale used. A similar result has been reported in human KB cells (29). No variation in the level of DNA polymerase-α was found after infection (Fig. 1, C2 and C3), in agreement with the results obtained above, using the nonfractionated cytoplasmic extracts (Table 1).

In the nuclei from growing cells (Fig. 1, N1), both DNA polymerases-α and -β (the latter sedimenting at 3.2*S*) were present, as was also found by Weissbach et al. (30) and Baril et al. (2). Infection by SV40 resulted in a threefold increase in the activity of both these enzymes, in agreement with the data shown in Table 1. No difference in this enhanced level of activity was found at the two times after infection (Fig. 1, N2 and N3).

The Triton wash of purified nuclei from growing cells contained a majority (83%) of the α-enzyme, together with some DNA polymerase-β (Fig. 1, T1). At 15 h postinfection, i.e., at the time when SV40 DNA synthesis is known to begin (18), the increased DNA polymerase activity detected in nonfractionated nuclear membrane extracts was mostly that of DNA polymerase-α (Fig. 1, T2). At 39 h postinfection, the activity of the enzyme was back to normal (Fig. 1, T3), confirming the results shown in Table 1.

It should be pointed out that, in all of these experiments, sedimentation analysis was performed under ionic-strength conditions that prevented aggregation forms of the enzymes (3, 29; our unpublished data obtained with purified DNA polymerases-α and -β from regenerating rat liver). Moreover, it was observed that the material sedimenting at the position of DNA polymerase-α in Fig. 1 retained its sedimentation properties when pooled and resedimented in sucrose gradients containing 0.4 M KCl (data not shown). Finally, DNA synthesis using RNA-primed DNA template was also tested in each of the sucrose gradient fractions: the activity was exactly and exclusively found at the position of the α-enzyme.

The RNA-dependent DNA polymerase activity of the cell extracts was resolved into two main peaks by sucrose gradient centrifugation (Fig. 2). One sedimented at 3.2*S*, corresponding to DNA polymerase-β (5), and the second sedimented at 6.5*S* to 7.5*S*, corresponding to DNA polymerase-γ. It is known that DNA polymerase-α which has an *S* value slightly higher, cannot copy an RNA template (3, 8, 27). The use of 40 mM potassium phosphate in the assay led to a clear distinction of the activity of DNA

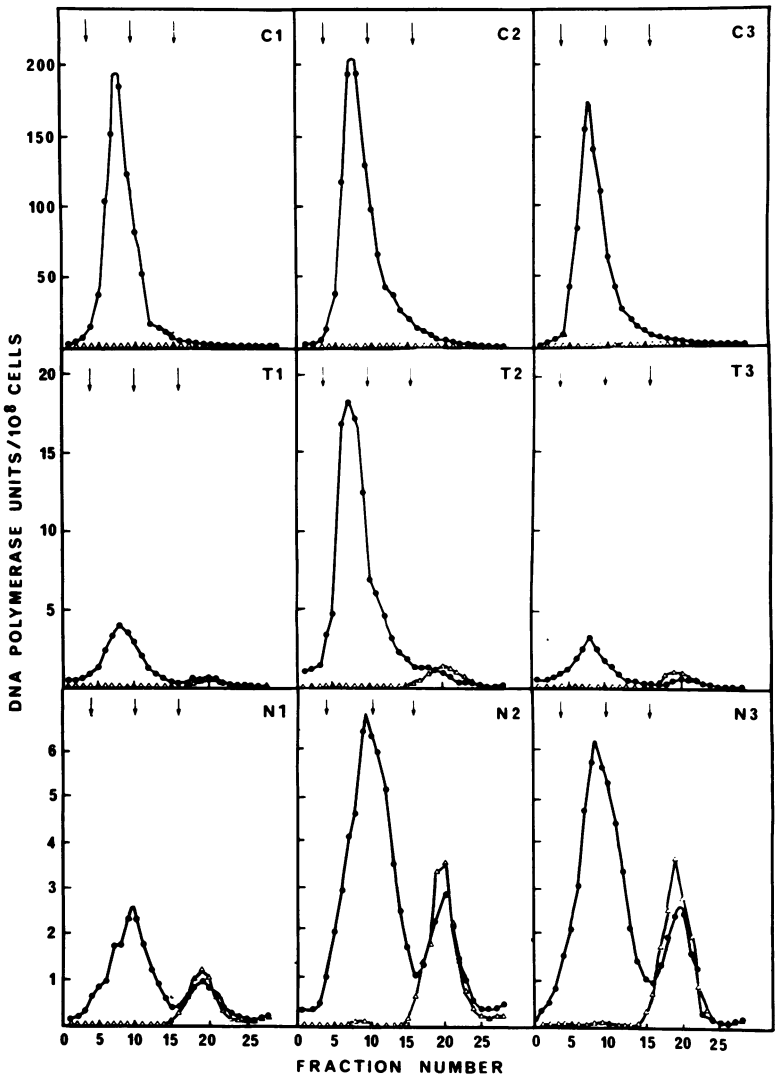

FIG. 1. *Sucrose gradient sedimentation of DNA-dependent DNA polymerase activities from cell fractions of SV40-infected growing cells. Preparation of cell extracts and sedimentation conditions were as described in the text. C, T, and N refer to cytoplasmic extracts, Triton X-100 washes of purified nuclei, and nuclear extracts, respectively. Numbers 1, 2, and 3 following C, T, and N refer to mock-infected growing cells and infected growing cells at 15 and 39 h postinfection, respectively. DNA polymerase activity was determined with poly(dC)·(dG)$_{12-18}$ in optimal conditions for DNA polymerase-α (●) and DNA polymerase-β (△), as described in the text. Arrows from left to right in each panel indicate the positions of catalase (11.3S), alcohol dehydrogenase (7.4S), and bovine serum albumin (4.4S) as markers run in parallel.*

polymerase-γ, which was stimulated by 26%, from that of β-polymerase, which was completely inhibited under these conditions (13). Poly(C)·(dG)$_{12-18}$ was not copied by the DNA polymerase-γ from the sucrose gradients in the high-salt conditions described by Knopf et al. (13), in contrast to what was found when purified reverse transcriptase from avian myeloblastosis virus (a gift from Boehringer Mannheim) was used.

In the cytoplasmic fraction, no significant variation of RNA-dependent DNA polymerase activities was found after infection (Fig. 2, C1, C2, and C3). In the nuclear membrane extract (Fig. 2, T1, T2, and T3), a slight increase in the activity of DNA polymerase-γ was noted at 15 h postinfection. On the other hand, nuclei from infected cells exhibited a DNA polymerase-γ activity enhanced by approximately 2.5-fold at both 15 and 39 h after infection (Fig. 2, N2 and N3). The enhanced activity detected at 3.2S corresponds to the increase in DNA polymer-

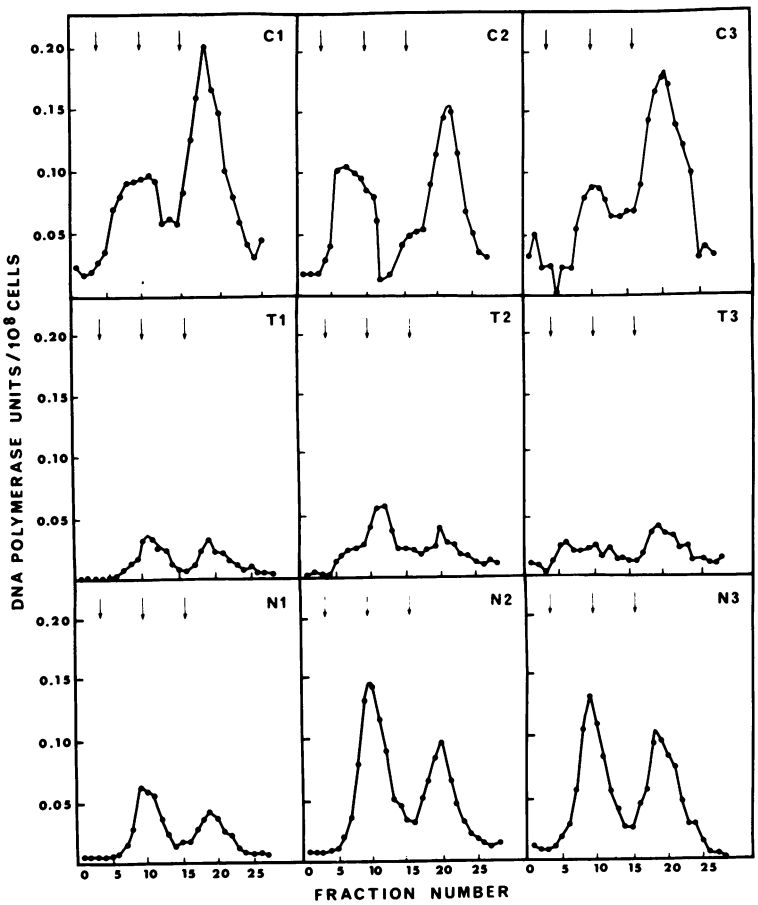

FIG. 2. *Sucrose gradient sedimentation of RNA-dependent DNA polymerase activities from cell fractions of SV40-infected growing cells. Analysis of DNA polymerase activities using poly(A)·(dT)$_{12-18}$ as a template was performed as described in the text. Panels C, T, and N and numbers 1, 2, and 3 are as described in the legend to Fig. 1. Arrows indicate the positions of catalase (11.3S), alcohol dehydrogenase (7.4S), and bovine serum albumin (4.4S) markers run in parallel.*

ase-β activity demonstrated previously (Fig. 1).

The total amount of enzyme activity in each of the peaks of the gradients shown in Fig. 1 and 2 is recorded in Table 2: early in infection the activity of DNA polymerase-α was increased approximately fourfold in the nuclear membrane extracts and threefold in the nuclear extracts. It then remained enhanced only inside the cell nuclei. That of DNA polymerase-β was increased approximately twofold in the nuclear membrane extract and threefold in the cell nuclei. Again, the increase was transient in the nuclear membrane extract, but stable in the nuclear fraction. The activity of DNA polymerase-γ, which in any case represented a very small amount of the total DNA polymerase activity of the cell extracts, varied somewhat like that of DNA polymerase-β. It is not known, however, whether this variation was significant, due to the low level of activity detected. It must be noted that, in some experiments, DNA polymerase-γ from infected cells was found to sediment at 11 to 12S in 0.1 M KCl, which was much faster than the α-enzyme (which aggregates at 10 mM KCl under our conditions). Resedimentation of the 12S enzyme at higher ionic strength (0.4 M KCl) yielded a peak of γ-activity at 7S, indicating that the γ-enzyme from infected cells was aggregated at the lower ionic strength. The nature of this aggregation has not been studied, but similar results have been reported by Matsukage et al. (19), Knopf et al. (13), and U. Bertazzoni (personal communication) with noninfected cell extracts.

DISCUSSION

The present study was undertaken to investi-

TABLE 2. *DNA polymerases-α, -β, and -γ separated by sucrose gradient sedimentation from SV40-infected cell extracts*[a]

Cells	DNA polymerase activity (U/10⁸ cells) in:								
	Cytoplasmic extract			Nuclear membrane extract			Nuclear extract		
	α	β	γ	α	β	γ	α	β	γ
Mock-infected	897	0.62	0.73	24	4.4	0.16	15.9	5.7	0.30
Infected at 15 h postinfection	1,019	0.53	0.64	101	8.1	0.33	47.7	16.5	0.73
Infected at 39 h postinfection	769	0.49	0.52	19	5.6	0.18	42.6	15.6	0.64

[a] DNA polymerases from the extracts in Table 1 were separated by sucrose gradient centrifugation (Fig. 1 and 2) and measured using poly(dC)·$(dG)_{12-18}$ for the α- and β-enzymes and poly(A)·$(dT)_{12-18}$ for the γ-enzyme (see text).

gate the influence of SV40 infection on the level and intracellular location of the three DNA polymerase activities present in growing cells, i.e., in cells already involved actively in DNA synthesis at the time of infection. Cell fractions investigated in these experiments were the cytoplasmic fraction, the nuclear membrane fraction, as obtained after treatment of purified nuclei with Triton X-100 (1), and the remaining cell nuclei.

In the cytoplasmic fraction, the amount of DNA polymerases-α, -β, and -γ remained unaltered after SV40 infection. This is different from the results obtained when resting cells infected by polyoma virus (21, 31) were used. The discrepancy between our results and those obtained with polyoma virus is probably due to the fact we used growing cells instead of resting cells. It is known that the activity of DNA polymerase-α is increased in the cytoplasm of cells entering the S phase (2, 3, 6, 7, 26, 29), and the enhanced level of DNA polymerase activity that is found in the cytoplasm of infected resting cells (21, 31) might therefore be linked only to the induction of cell DNA synthesis after infection and not to the actual replication of the viral genome.

The results obtained with the nuclear fractions show that the activity of all three DNA polymerases-α, -β, and -γ was increased in the cell nuclei after SV40 infection, at early as well as late times after infection (Fig. 1 and 2). It is not known whether this reflects the SV40 DNA elongation process of initiated DNA molecules or the fact that SV40 infection increases host cell DNA repair, particularly at 39 h postinfection.

On the other hand, SV40 infection of growing cells induced a marked increase in the activity of DNA polymerase-α in the nuclear Triton wash early in infection. It was previously suggested that the cellular event that is required for the initiation of SV40 DNA replication might be the formation of specific replication sites in the infected cell (22). Leblanc and Singer have shown that early in infection most of the replicating DNA molecules are recovered in association with host nuclear membrane components, as isolated by the M band technique (14). Although strict criteria for the definition and isolation of a membrane-DNA complex are not yet clearly defined, the early stages of SV40 DNA replication might occur at the nuclear membrane. In this respect, it is of great interest to note that the DNA polymerase activity that was the most enhanced at 15 h after infection in the cell fractions tested was that of DNA polymerase-α from the nuclear membrane extract and that this activity was back to normal at 39 h after infection, i.e., at the time when the percentage of replicated molecules that became replicative has decreased (18). It would be tempting to associate the polymerase activities detected in the nuclear Triton wash fraction with nuclear membrane-associated polymerase activities. However, as indicated above, in addition to the polypeptides of the nuclear membranes (1), proteins of cytoplasmic origin migrating toward the nucleus (or inversely) might be found in this extract, and thus the significance of the polymerase activities removed from purified nuclei by the Triton wash is still unclear. Intracellular migration of DNA polymerase from the cytoplasm to the nucleus has been suggested in polyoma-infected cells (31), and the enhancement of DNA polymerase-α activity in the Triton wash fraction that occurs early after SV40 infection or in regenerating rat liver (7) may reflect this phenomenon without a necessary nuclear membrane association.

DNA polymerase-α is the only animal DNA polymerase known to be able to catalyze an RNA-primed DNA synthesis (8, 27), and our results clearly show that such activity was greatly enhanced in the infected cell extracts

(Table 1 and Fig. 1). Since RNA primers have been involved in viral DNA replication (16, 17, 23), these results suggest altogether that DNA polymerase-α is involved in SV40 DNA replication. We cannot, however, exclude the possibility that new cell DNA replicons are induced by SV40 infection even in cells already actively engaged in replication, since the ratio of total cell DNA content (as determined above) of infected to mock-infected growing cells was 1.30 in these experiments.

Finally, it should be stressed that the absolute levels of DNA polymerase-γ measured in these experiments should be considered carefully. DNA polymerase-γ activity was mostly found in the cytoplasm and washed nuclei, but the amount of activity recovered was very low compared with that of DNA polymerases-α and -β found with HeLa cells (26). Although it is known that purified DNA polymerase-γ copies poly(A) 20 times more rapidly than does activated DNA (13, 26), it is not certain that in vitro assay conditions are the best for testing this enzyme. Still, even a limited amount of this enzyme could be sufficient to play a prominent role in the DNA replication process. The fact that DNA polymerase-γ activity was enhanced in cell nuclei after infection does not, however, allow the resolution of this question at the moment.

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