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Role of DNA polymerase α and DNA primase in simian virus 40 DNA replication *in vitro*

(large tumor antigen/eukaryotic DNA replication)

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ABSTRACT The role of DNA polymerase α (pol α) and DNA primase has been investigated in the simian virus 40 (SV40) DNA replication system *in vitro*. Removal of pol α and primase activities from crude extracts of HeLa cells or monkey cells by use of an anti-pol α immunoaffinity column resulted in the loss of replication activity. The addition of purified pol α–primase complex isolated from HeLa cells or monkey cells restored the replication activity of depleted extracts. In contrast, the pol α–primase complex isolated from either mouse cells or calf thumus did not. Extracts prepared from mouse cells (a source that does not support replication of SV40) did not replicate SV40 DNA. However, the addition of purified pol α–primase complex isolated from HeLa cells activated mouse cell extracts. pol α and primase from HeLa cells were extensively purified and separated by a one-step immunoaffinity adsorption and elution procedure. Both activities were required to restore DNA synthesis; the addition of pol α or primase alone supported replication poorly. Crude extracts of HeLa cells that were active in SV40 replication catalyzed the synthesis of full-length linear double-stranded (RFIII) DNA in reaction mixtures containing poly(dT)-tailed pBR322 RFIII. Maximal activity was dependent on the addition of oligo(dA), ATP, and creatine phosphate and was totally inhibited by aphidicolin. Since pol α alone could not replicate this substrate and since there was no degradation of input DNA, we propose that other enzymatic activities associate with pol α, displace the non-template strand, and allow the enzyme to replicate through duplex regions.

The replication of simian virus 40 (SV40) DNA is an important model system for studying eukaryotic DNA replication because it requires only one virus-encoded protein, the SV40 large tumor antigen (T antigen); all the other components involved in this process are supplied by host cells. Our interest is in the isolation of factors that are involved in mammalian DNA replication. For this purpose, we have developed an *in vitro* replication system that is analogous to those described by others (1–3), using a salt extract of exponentially growing HeLa cells and plasmid DNA containing the SV40 origin sequence (4, 5). Replication with these extracts was shown to be totally inhibited by aphidicolin, a specific inhibitor of DNA polymerase α (pol α). In this communication we describe the role of pol α and DNA primase in the replication system. pol α plays a key role in mammalian DNA replication (6–8). We have found that pol α and primase activity are essential for the *in vitro* replication of DNA containing the SV40 origin. We have also found that the cellular source of these activities plays an important role in determining whether SV40 DNA replication can be catalyzed by extracts *in vitro*. Those cells that support replication *in vivo* yield extracts that replicate SV40 DNA *in vitro*; cells that do not support replication yield extracts that are inactive. The pol α–primase complex isolated from permissive cells supported replication in extracts depleted of these activities, whereas the complex isolated from nonpermissive cells was inactive.

In addition, we have developed a replication assay with which we can independently study the elongation step of DNA replication using linear duplex DNA with long single-stranded tails at its 3′ termini.

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MATERIALS AND METHODS

***In Vitro* Replication System for SV40 ori$^+$ DNA.** The reaction conditions and methods used for the preparation of extracts of HeLa cells (4) and SV40 T antigen (9) have been described. The extracts from FM3A mouse cells and monkey COS cells were prepared the same way as extracts from HeLa cells. Reaction mixtures (50 μl) contained 30 mM Tris·HCl buffer (pH 8.5); 7 mM $MgCl_2$; 0.5 mM dithiothreitol; 4 mM ATP; 200 μM each CTP, UTP, and GTP; 100 μM each dATP, dCTP, and dGTP; 25 μM [*methyl*-^{3}H]dTTP (300 cpm/pmol); 40 mM creatine phosphate; 1 μg of creatine kinase, 0.3 μg of superhelical circular duplex (RFI) plasmid (pSV01ΔEP3, ori$^+$); 200–400 μg of HeLa extract; and 0.5–1 μg of T antigen. The ori$^+$ DNA contained the 311-base-pair (bp) origin of replication (the *Eco*RII fragment G of SV40 DNA) inserted into a 2481-bp pBR322 derivative as described (4). Reaction mixtures were incubated at 37°C as indicated and the radioactivity incorporated into acid-insoluble material was measured. When products obtained in the replication reaction were analyzed, [α-^{32}P]dCTP (3000 cpm/pmol) was used and analysis was carried out by agarose gel electrophoresis followed by autoradiography.

Depletion of pol α–Primase Complex. An anti-pol α antibody column was prepared using SJK-287-37 hybridoma [American Type Culture Collection (10)] supernatant and anti-mouse IgG-Sepharose as described (11, 12). The culture supernatant (300 ml) was used to prepare a 5-ml antibody column. A crude extract (100 mg of protein) from HeLa cells (4) was adjusted to 0.35 M NaCl and applied to a 2-ml column. The flow-through fraction was dialyzed against buffer A [20 mM Tris·HCl, pH 8.5/1 mM EDTA/1 mM dithiothreitol/10% (vol/vol) glycerol/50 mM NaCl] and was used as the pol α–primase-depleted extract.

Purification of pol α–Primase Complex. A crude extract from HeLa cells (4) (prepared from 10^{10} cells), adjusted to 250 mM NaCl in buffer B (20 mM Tris·HCl, pH 8.5/1 mM EDTA/1 mM dithiothreitol/20% glycerol), was applied to a

Abbreviations: SV40, simian virus 40; T antigen, SV40-encoded large tumor antigen; RFI, superhelical circular duplex DNA; RFII, circular duplex DNA containing at least one single-strand break; RFIII, linear product formed from RFI DNA; pol α, DNA polymerase α; u, unit(s).

DEAE-cellulose column (100 ml) equilibrated with buffer B plus 250 mM NaCl. The flow-through fraction was dialyzed against buffer B containing 50 mM NaCl and applied to a second DEAE-cellulose column (50 ml) equilibrated with buffer B containing 50 mM NaCl. The pol α–primase complex was eluted by the addition of buffer B containing 250 mM NaCl. The eluate (32 ml) was directly applied to a phosphocellulose column (50 ml) equilibrated with buffer B plus 150 mM NaCl. The column was subjected to gradient elution (0.15–1 M NaCl linear gradient, 300 ml). pol α and primase were co-eluted between 300 mM and 500 mM NaCl. The active fractions were pooled and dialyzed against buffer B plus 50 mM NaCl and loaded onto a single-stranded DNA-cellulose column (20 ml) prewashed with buffer B containing 50 mM NaCl. The column was developed with a 120-ml linear gradient of 50–500 mM NaCl in buffer B; the enzyme activities were recovered between 75 mM and 150 mM NaCl. Active fractions were directly applied to a 1-ml phosphocellulose column and eluted with 4 ml of buffer B containing 1 M NaCl. The eluate was dialyzed against buffer B containing 50 mM NaCl and used as the purified complex. Mouse pol α–primase complex was purified from 6×10^9 cells [mammary carcinoma FM3A c128 (13)] and monkey pol α–primase complex was purified from 10^9 COS-1 cells, using the procedure described above. The specific activities of these enzymes in the assays described below were as follows: preparations from HeLa cells, 0.20 unit (u) of pol α and 0.50 u of primase per μg of protein; from FM3A cells, 0.60 u of pol α and 0.41 u of primase per μg; from COS-1 cells, 0.25 u of pol α and 0.55 u of primase per μg. Calf thymus enzyme was purchased from Pharmacia and contained 0.14 u of pol α and 0.05 u of primase per μg.

Separation of pol α and Primase. The separation of HeLa cell primase activity and pol α was carried out as follows. Crude extract (75 mg of protein) of HeLa cells (4), adjusted to 0.35 M NaCl in buffer C (20 mM Tris·HCl, pH 8.5/1 mM EDTA/1 mM dithiothreitol/10% glycerol), was applied to a 2-ml antibody column, containing anti-pol α monoclonal antibody (from hybridoma SJK-237) and equilibrated with buffer C plus 0.35 M NaCl. After washing with 6 ml of the starting buffer, both activities were eluted separately by the stepwise addition of 37% and 50% (vol/vol) ethylene glycol in buffer C plus 0.5 M NaCl (6 ml each). The separated fractions were dialyzed against buffer C containing 50 mM NaCl. This procedure yielded primase (6 u/μg of protein) and pol α (20 u/μg of protein) preparations that were virtually free of cross-contamination (<2%).

The mouse pol α–primase complex was resolved into its individual activities (14) as follows. The purified complex (4 ml), prepared as described above, was dialyzed against buffer C containing 50% ethylene glycol and 50 mM NaCl and then was applied to a 2-ml DEAE-cellulose column equilibrated with buffer C containing 50% ethylene glycol and 0.25 M NaCl. The primase activity was recovered in the flow-through fraction, and pol α activity was eluted by the stepwise addition of 6 ml of buffer C containing 50% ethylene glycol and 0.25 M NaCl. The specific activity of each enzyme did not change in this step.

Assay systems for pol α and primase have been described (15). The reaction mixture (50 μl) for the pol α assay contained 50 mM Tris·HCl (pH 7.9); 7.5 mM $MgCl_2$; 4 mM dithiothreitol; 20 μg of bovine serum albumin (BSA); 40 μM each dATP, dCTP, and dGTP; 8 μM [^{3}H]dTTP (100 cpm/pmol); and 10 μg of activated calf thymus DNA (16). The reaction mixture (50 μl) for the primase assay contained 50 mM Tris·HCl (pH 7.9); 4 mM dithiothreitol; 9 mM $MgCl_2$; 10 μg of BSA; 1 nmol of poly(dT) (P-L Biochemicals); 8 μM [^{3}H]dATP; 5 mM ATP; and 0.5 u of DNA polymerase I (Boehringer Mannheim). One unit of pol α and one unit of primase incorporated 1 nmol of dTMP and 1 nmol of dAMP, respectively, after incubation at 30°C for 30 min.

Preparation of Poly(dT)-Tailed pBR322 RFIII. pBR322 RFI DNA was linearized with *Pvu* I (New England Biolabs) under the supplier's recommended conditions and 0.2 volume of "5 × TdT tailing buffer" (Bethesda Research Laboratories), 50 μM [*methyl*-^{3}H]dTTP (Amersham), and 22 u of terminal deoxynucleotidyltransferase (Pharmacia) were added. Incubation was continued at 37°C for 4 hr. The DNA was then extracted with phenol/chloroform (1:1), ethanol-precipitated, dissolved in 10 mM Tris Cl, pH 8.0/1 mM EDTA, and stored at 4°C. An average of 250 pmol of dTMP was incorporated per pmol of 3′ terminus.

RESULTS

The Role of pol α and Primase in SV40 ori⁺ Replication. In order to study the role of pol α and primase, pol α–primase-depleted extracts were prepared by passing the crude extract from HeLa cells through an anti-pol α immunoaffinity column. This procedure removed about 70% of pol α activity and 90% of primase activity present in the extract. Further recycling of the depleted extract through immunoaffinity columns did not remove the residual pol α activity. When the depleted extract was examined in the SV40 replication system, it was inactive (Fig. 1*A*). This suggested that the pol α–primase complex is essential for replication. This was verified by the reconstitution of the replication activity with purified pol α–primase complex isolated from HeLa cells (Fig. 1*A*). The extent of replication depended upon the amount of pol α–primase activity added; all incorporation with the reconstituted system required T antigen and DNA containing the SV40 origin. The pol α–primase complex purified from monkey COS cells was as active as that from HeLa cells. In contrast, the complex purified from mouse FM3A cells or calf thymus did not restore the replication activity (Fig. 1*A*). The combination of the pol α–primase

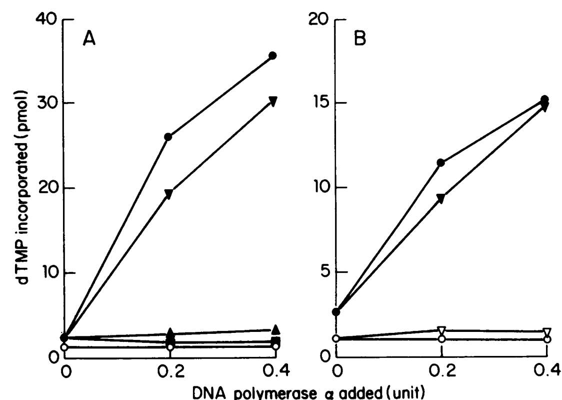

FIG. 1. Reconstitution of DNA replication by pol α–primase-depleted HeLa cell extract by the addition of pol α–primase complex isolated from various sources. (*A*) Reconstitution using HeLa cell depleted extract. Various amounts of pol α–primase complex from HeLa cells (●), FM3A mouse cells (▲), COS cells (▼), and calf thymus (■) were added to the pol α–primase-depleted extract of HeLa cells. Incubations were carried out in the presence of T antigen (solid symbols) or in the absence of T antigen (open symbols) for 90 min at 37°C, and the amount of acid-insoluble radioactivity was determined. In each assay, 250 μg of protein was used. Under these conditions, the original HeLa cell extract catalyzed the incorporation of 76.8 pmol of dTMP after 90 min at 37°C. (*B*) Reconstitution using COS cell depleted extract. Conditions were as in *A*, except that the pol α–primase-depleted extract (250 μg of protein per assay) was isolated from COS cells. Symbols used are the same as in *A*. Under these conditions, the COS cell extract prior to depletion catalyzed the incorporation of 34 pmol of dTMP.

complex from thymus or mouse cells with the complex from active sources did not inhibit the latter. The pol α and DNA primase activities in extracts from monkey cells were also removed by immunoaffinity columns. These depleted monkey extracts supported SV40 replication when supplemented with the pol α–primase complex isolated from either HeLa cells or monkey cells (Fig. 1B).

Activation of Mouse Cell Extract by HeLa pol α–Primase Complex. The experiments described above were carried out with the pol α–primase-depleted extract from cell extracts that supported SV40 replication. Monkey cells and human cells are known to be permissive for the DNA replication of SV40 *in vitro* (17) as well as *in vivo* (18). In contrast, mouse cells are nonpermissive for DNA replication of SV40 but are permissive for the DNA replication of polyoma virus. To determine what factor(s) is responsible for the permissiveness in the SV40 DNA replication *in vitro*, we prepared extracts from mouse FM3A cells and tested various fractions obtained from HeLa cell extracts. As shown in Fig. 2, purified pol α–primase complex isolated from HeLa cells activated mouse cell extracts which were inactive by themselves. The addition of mouse pol α–primase complex had no effect. The activation was dependent upon the presence of T antigen and DNA containing the SV40 origin (Fig. 3, lanes 10 and 11). In addition, replication products formed were the same as those synthesized by extracts of HeLa cells (Fig. 3, lanes 4 and 12). These results suggest that the pol α–primase complex plays an important role in the determination of permissiveness of SV40 DNA replication *in vitro*. This is consistent with earlier results showing permissiveness is due to *trans*-acting factors required for viral DNA replication of SV40 (19).

Reconstitution of Replication Activity with Separated pol α and Primase Activities. As described in the previous section, the replication reaction with depleted extract was restored by the addition of the HeLa cell pol α–primase complex. We examined whether both pol α and primase activities were essential for replication. For this purpose, HeLa pol α activity was separated from primase activity by use of an anti-pol α antibody column. After the application of the HeLa crude extract to the column, primase and pol α were eluted individually (Fig. 4). Both enzymes were highly purified by this one-step procedure. These isolated activities were added to depleted extracts and assayed for replication activity (Table 1). The combination of HeLa cell pol α and Hela cell primase supported replication, whereas the addition of either enzyme alone did not. These results suggest that both pol α and primase activities are essential for replication. We also carried out the reconstitution experiment using mouse pol α and mouse primase in conjunction with the HeLa enzymes. None of the mouse enzymes worked when complemented with the HeLa enzyme (Table 1). This suggests that both the pol α and the primase from a permissive cell are essential for replication. Neither mouse pol α nor mouse primase preparation inhibited the replication carried out with the HeLa cell enzymes (unpublished results), thus demonstrating the absence of inhibitors in the mouse fractions.

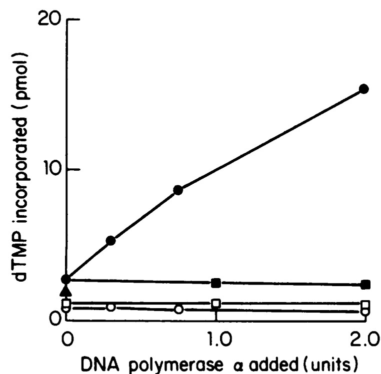

FIG. 2. Activation of mouse cell extracts with purified pol α–primase. Various amounts of purified pol α–primase complex from HeLa (●, ○) or mouse FM3A (■, □) cells were added to mouse FM3A extract (250 μg of extract protein). Reactions were carried out in the presence of T antigen (solid symbols) or in the absence of T antigen (open symbols). After a 2-hr incubation, acid-insoluble radioactivity was determined.

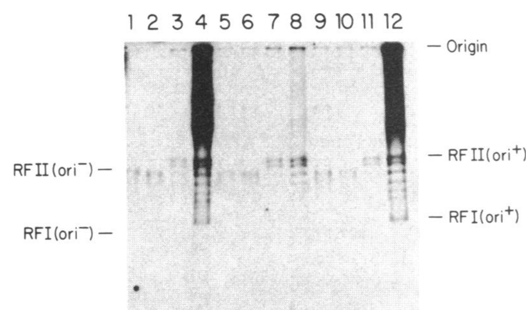

FIG. 3. Analysis of DNA products formed by various cell extracts and HeLa cell pol α–primase. Reaction mixtures contained HeLa cell extract (lanes 1–4) or mouse cell extract (lanes 5–12) with (lanes 2, 4, 6, 8, 10, and 12) or without (lanes 1, 3, 5, 7, 9, and 11) SV40 T antigen, using DNA with (lanes 3, 4, 7, 8, 11, and 12) or without the SV40 origin sequence (lanes 1, 2, 5, 6, 9, and 10). Half the reaction mixtures containing mouse cell extract (lanes 9–12) were supplemented with HeLa pol α–primase (1 u of pol α and 2.5 u of primase). RFI (replicative form I), superhelical circular duplex DNA; RFII, circular duplex DNA containing at least one single-strand break.

Replication of Poly(dT)-Tailed pBR322 RFIII. The initiation reaction with SV40 ori⁺ DNA is dependent on the T antigen. Primase and pol α play an important role in this event. However, once DNA chains have been started, pol α must participate in an elongation reaction. Although pol α can extend 3′-hydroxyl ends hydrogen-bonded to a single-stranded DNA template, it cannot extend primer ends that are juxtaposed to a duplex structure. These findings suggest that auxilliary factors must act conjointly with pol α.

The fractions that support SV40 replication were examined for their ability to support elongation of primed templates through duplex DNA. For this purpose, we added poly(dT) chains at each 3′ terminus of pBR322 RFIII DNA. Under conditions similar to those used for the *in vitro* replication reaction (see above), HeLa extracts catalyzed the incorporation of dCMP into pBR322 RFIII that contained single-

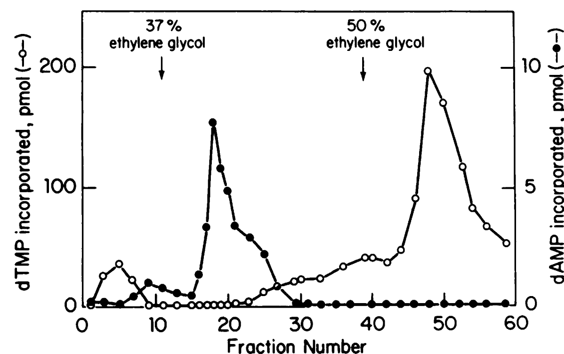

FIG. 4. Separation of HeLa pol α–primase complex into pol α and primase fractions. HeLa cell crude extract (75 mg of protein) was applied to an anti–pol α column. Primase (●) was eluted with 37% ethylene glycol in buffer C and pol α (○) was eluted with 50% ethylene glycol in buffer C. Fractions 17–21 and fractions 46–53 were pooled, dialyzed, and used as such.

Table 1. Reconstitution of the replication activity with pol α and primase from HeLa cells or from mouse FM3A cells

Enzyme(s) added	[^{3}H]dTMP incorporated, pmol
HeLa pol α (0.4 u)	1.25
HeLa primase (0.5 u)	0.36
HeLa pol α (0.4 u) + HeLa primase (0.5 u)	16.7
FM3A pol α (0.4 u)	0.80
FM3A primase (0.5 u)	0.05
HeLa pol α (0.4 u) + FM3A primase (0.5 u)	0.60
FM3A pol α (0.4 u) + HeLa primase (0.5 u)	0.60
FM3A pol α (0.4 u) + FM3A primase (0.5 u)	0.05

Reaction mixtures (50 μl) were as described in *Materials and Methods*, with 0.3 μg of pSV01ΔEP (ori$^+$DNA), 250 μg of pol α–primase-depleted HeLa, and 0.5 μg of T antigen. The amount of purified enzyme(s) added to the depleted extract is indicated in parentheses. Acid-insoluble radioactivity was determined after 2 hr of incubation at 37°C. The depleted extract incorporated 1.21 pmol under the conditions described above. This number has been subtracted from those presented above.

stranded poly(dT) tails at its 3′ termini. This reaction was dependent upon the presence of the poly(dT) tails and was markedly stimulated by the addition of oligo(dA) primers (Table 2). The reaction was inhibited by 0.1 mM aphidicolin [a concentration sufficient to selectively inhibit pol α (20, 21)], but purified pol α–primase alone could not catalyze this reaction. ATP and an ATP-regenerating system were also required (Table 2). The poly(dT) tails were not degraded (<5% of [^{3}H]dTMP was recovered in acid-soluble material after a 30-min incubation), nor was the ^{32}P-labeled DNA product after phenol extraction and reincubation with the HeLa extract (unpublished observations).

Polyacrylamide gel electrophoresis under denaturing conditions showed that the products were apparently full-length (Fig. 5, lane 1). To determine whether this was due to repair synthesis, the DNA was cut with *Dpn* I, which will cleave only if both adenosine residues in its recognition sequence (GATC) are methylated. Since the pBR322 DNA was maintained in a *dam*$^+$ strain of *Escherichia coli* (HB101), products arising from short-patch repair-type synthesis would be degraded; products of full-length replication should remain intact. As shown in Fig. 5, lane 3, the products were largely resistant to *Dpn* I but sensitive to *Sau*3A1 (lane 2), which cuts at the same sequence but is insensitive to the methylation state of the DNA. The products were resistant to *Mbo* I (unpublished observation), an enzyme that cleaves only fully unmethylated GATC sites, indicating that progeny molecules were not utilized for multiple rounds of replication.

Table 2. Requirements for replication of poly(dT)-tailed pBR322 RFIII

Conditions	dCMP incorporated, fmol/30 min
Complete	363
Omit extract	<1
Omit DNA	3
Omit oligo(dA)	86
Omit DNA, add pBR322 RFIII without poly(dT) tails	36
Omit extract, add pol α–primase complex	20
Omit ATP, creatine phosphate, and creatine kinase	13
Add aphidicolin (0.1 mM)	8

Complete reaction mixtures (25 μl) contained 30 mM Tris·HCL (pH 8.5); 7 mM $MgCl_2$; 0.5 mM dithiothreitol; 4 mM ATP; 200 μM CTP, GTP, and UTP; 100 μM dATP, dCTP, and dTTP; 0.5 μM [α-^{32}P]dCTP (800 cpm/fmol, Amersham); 40 mM creatine phosphate; 1 μg of creatine kinase; 50 ng of poly(dT)-tailed pBR322 RFIII; 50 pmol of $(dA)_{12-18}$ (P-L Biochemicals); and 100 μg (protein) of HeLa cell extract. After incubation for 30 min at 37°C, acid-insoluble radioactivity was measured. Where indicated, HeLa pol α–primase complex (1 u of pol α and 2.5 u of primase) was added.

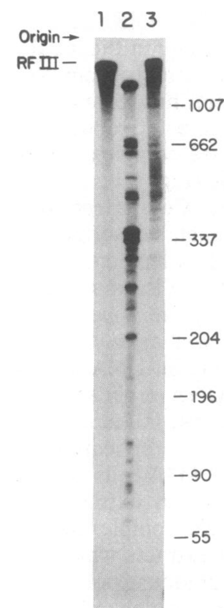

FIG. 5. Analysis of product formed in replication assay using poly(dT)-tailed pBR322 DNA. Replication assays were carried out as described in the legend to Table 2; terminated by the addition of 0.5% $NaDodSO_4$, 10 mM EDTA, and 10 μg of yeast tRNA; and digested with proteinase K (200 μg/ml) for 30 min at 37°C. Products were then isolated by phenol extraction and ethanol precipitation, collected in 80% formamide, heated to 90°C for 3 min, and, after the treatments given below, subjected to electrophoresis in a 3.5% polyacrylamide gel [30:1 (wt/wt) acrylamide/*N, N*′-methylenebis(acrylamide)] containing 8 M urea. Lanes: 1, no treatment; 2, digestion with 1 unit of *Sau*3A1; 3, digestion with 1 unit of *Dpn* I. Restriction digestions were performed under the suppliers' recommended conditions. Prior to electrophoresis, the restriction enzyme-digested samples were phenol-extracted and ethanol-precipitated a second time. Numbers at right indicate the length of DNA markers in base pairs.

DISCUSSION

We have used an anti-pol α monoclonal antibody to analyze the role of pol α and primase in the replication of DNA containing the SV40 origin. Since primase forms a tight complex with pol α in higher eukaryotes (15, 22), this procedure also removed primase. The depleted extracts were inactive in the replication reaction. The addition of purified pol α–primase complex restored this activity. In contrast, pol α devoid of primase activity did not support replication; primase, containing no pol α, also did not restore replication activity. The combination of pol α and primase activities worked well, indicating that both enzymes are essential for replication.

The depleted system enabled us to test different pol α–primase-complex preparations for their ability to support replication. Pol α–primase complex purified from exponentially growing mouse cells and from calf thymus, neither of which is a natural host for the propagation of SV40 virus, were inactive, suggesting that the enzymes from cells that can support replication of SV40 DNA are required. In accord with this idea, the pol α–primase complex purified from monkey cells, a natural host for SV40, could reactivate the

depleted extract. Human cells are not natural hosts for SV40 virus, but they are permissive for replication of SV40 DNA (17, 18). Therefore, it is possible that the permissiveness of a given cell centers on the ability of T antigen to interact with the cellular pol α-primase complex, as well as on the ability of pol α-primase to interact with other cellular components. To test this possibility, we tried to activate mouse cell extracts (inactive by themselves) with various fractions obtained from HeLa cell extracts. pol α-primase-depleted extract failed to activate mouse cell extract (unpublished result). However, the addition of purified HeLa cell pol α-primase fractions to mouse extracts resulted in the replication of SV40 ori$^+$ DNA. This suggests that the pol α-primase complex plays a key role in determining whether a particular cell supports SV40 replication. It also suggests that most of the replication factors in the mouse-cell extract can work in the *in vitro* replication of SV40 DNA. The finding that pol α and primase must both be derived from a permissive cell suggests that the specificity resides in the complex.

In addition to the replication assay described above, we have also developed an elongation assay utilizing linear duplex DNA with primed single-stranded tails, a substrate resembling the leading strand of a replication fork. Nucleotide incorporation with this substrate was not as efficient as with the SV40 replication system. This elongation reaction was clearly dependent upon the presence of single-stranded DNA and an oligonucleotide primer, and the sensitivity to aphidicolin suggests that pol α is involved. Since pol α alone (or with primase) is incapable of replicating through duplex regions of DNA (Table 2 and ref. 23), the extract must contain factors that can remove this impediment, either nucleolytically or by strand-displacement. Since the substrate DNA, as well as the products, was not degraded under our replication conditions (see *Results*), we suggest that the latter is the case. Similar substrates have been used to study the ability of *E. coli* helicase II (24) and the adenovirus DNA-binding protein (unpublished results) to stimulate replication by their cognate DNA polymerases, presumably by strand-displacement.

In this communication, we have identified pol α and primase as factors that are essential for SV40 DNA replication *in vitro*. In addition to these proteins, we have resolved multiple fractions, all of which are required for replication (11). It is clear that the SV40 system is amenable to purification and will provide important insights into chromosomal replication.

Note Added in Proof. We have recently developed an *in vitro* system that supports the replication of polyoma virus DNA in the presence of polyoma large T antigen. In this system, extracts of HeLa cells were inactive, whereas extracts of mouse cells fully supported DNA replication (Y.M., T. Eki, C. Prives, M. Yamada, and J.H., unpublished observations).

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1. Ariga, H. & Sugano, S. (1983) *J. Virol.* **48**, 481–491.
2. Li, J. J. & Kelly, T. J. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6973–6977.
3. Stillman, B. W. & Gluzman, Y. (1985) *Mol. Cell. Biol.* **5**, 2051–2060.
4. Wobbe, C. R., Dean, F. B., Weissbach, L. & Hurwitz, J. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5710–5714.
5. Dean, F. B., Wobbe, C. R., Weissbach, L., Murakami, Y. & Hurwitz, J. (1985) in *Papilloma Viruses: Molecular and Clinical Aspects*, ed. Howlly, P. M. & Broker, T. M. (Liss, New York), pp. 397–406.
6. Sugino, A. & Nakayama, K. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7049–7053.
7. Murakami, Y., Yasuda, H., Miyasawa, H., Hanaoka, F. & Yamada, M. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1761–1765.
8. Murakami, Y., Eki, T., Hanaoka, F., Enomoto, T., Miyazawa, H. & Yamada, M. (1986) *Exp. Cell. Res.* **163**, 135–142.
9. Simanis, V. & Lane, D. P. (1985) *Virology* **144**, 88–105.
10. Tanaka, S., Hu, S. Z., Wang, T. S. F. & Korn, D. (1982) *J. Biol. Chem.* **257**, 8386–8390.
11. Murakami, Y., Wobbe, C. R., Dean, F. B., Weissbach, L. & Hurwitz, J. (1986) in *Cancer Cells—4 DNA Tumor Viruses: Control of Gene Expression and Replication* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), in press.
12. Chang, L. M. S., Rafter, E., Angle, C. & Bollum, F. J. (1984) *J. Biol. Chem.* **259**, 14679–14687.
13. Nakano, N. (1966) *Tohoku J. Exp. Med.* **88**, 69–84.
14. Suzuki, M., Enomoto, T., Hanaoka, F. & Yamada, M. (1985) *J. Biochem.* **98**, 581–584.
15. Gronostajski, R., Field, J. & Hurwitz, J. (1984) *J. Biol. Chem.* **259**, 9479–9486.
16. Aposian, C. M. & Kornberg, A. (1962) *J. Biol. Chem.* **237**, 519–525.
17. Li, J. J. & Kelly, T. J. (1985) *Mol. Cell. Biol.* **5**, 1238–1246.
18. Ozer, H. L., Slator, M. L., Dermody, J. J. & Mandel, M. (1981) *J. Virol.* **39**, 481–489.
19. Botchan, M., Topp, W. & Sambrook, J. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 709–719.
20. Ikegami, S., Taguchi, T., Ohashi, M., Oguro, M., Nagano, H. & Mano, Y. (1978) *Nature (London)* **275**, 458–460.
21. Huberman, J. A. (1981) *Cell* **23**, 647–648.
22. Conaway, R. C. & Lehman, I. R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2523–2527.
23. Weaver, D. T. & DePamphilis, M. L. (1982) *J. Biol. Chem.* **257**, 2075–2086.
24. Kuhn, B. & Abdel-Monem, M. (1982) *Eur. J. Biochem.* **125**, 63–68.