Mouse DNA Primase Plays the Principal Role in Determination of Permissiveness for Polyomavirus DNA Replication

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We have investigated the species-specific replication of polyomavirus DNA in the cell-free system that was established previously (Y. Murakami, T. Eki, M. Yamada, C. Prives, and J. Hurwitz, Proc. Natl. Acad. Sci. USA 83:6347–6351, 1986). Extracts from various species of cells supported polyomavirus DNA replication in a species-specific manner that was consistent with the host range specificitiy of polyomavirus; extracts prepared from mouse and hamster cells were active, whereas extracts prepared from human, monkey, and insect cells were inactive. The addition of DNA polymerase α-primase purified from mouse cells induced the replication of polyomavirus DNA in a cell-free system containing polyomavirus large tumor antigen and nonpermissive cell extracts, such as human and insect cell extracts. Isolated mouse DNA primase alone also induced polyomavirus DNA replication in human cell extracts but not in insect cell extracts, indicating that mouse DNA primase plays the principal role in determining permissiveness for polyomavirus DNA replication.

To understand the molecular mechanism of DNA replication in mammalian cells, it is important to establish a simple model system that permits the analysis of DNA replication at the molecular level. The replication of simian virus 40 (SV40) DNA is an important model system for eukaryotic DNA replication because it requires only one virus-encoded protein, large tumor antigen (T antigen), and all other components involved in the DNA replication are supplied by host cells (reviewed in references 6, 10, 13, and 33). In this context, cell-free DNA replication systems consisting of human or monkey cell extracts, SV40 T antigen, and DNA containing the SV40 origin sequence have been established (2, 19, 29, 37) and have contributed to the identification and functional characterization of components of the cellular replication machinery (reviewed in references 16 and 17).

A cell-free replication system for mouse polyomavirus (PyV) DNA also has been developed, and this system consists of DNA containing the PyV origin sequence, PyV T antigen, and mouse cell extracts (24). Analysis of the cell-free replication systems for SV40 and PyV DNAs revealed that the replication of both viral DNAs required cell extracts of specific species (20, 24, 25, 36, 38). This species specificity is consistent with the permissiveness of host cells; monkey and human cells support the propagation of SV40 but not PyV, whereas mouse cells replicate PyV but not SV40.

It has been observed that hybrid cells formed between permissive and nonpermissive cells yield viral progeny (33, 35). Thus, it is likely that permissiveness is determined by transacting dominant factors present only in permissive cells. In fact, Murakami et al. (25) observed that the addition of a purified DNA polymerase α-primase complex isolated from HeLa cells activated mouse cell extracts to replicate SV40 DNA. In addition, PyV DNA synthesis was restored in HeLa cell extracts when a purified mouse DNA polymerase α-primase complex was added to the extracts (24).

In this study, we have analyzed the species specificity of PyV DNA replication in detail by using cell extracts of various species, purified DNA polymerase α-primase, isolated DNA polymerase α, and isolated DNA primase and have presented evidence indicating that DNA primase plays the principal role in the determination of permissiveness of cell extracts for PyV DNA replication.

MATERIALS AND METHODS

Materials. Phosphocreatine kinase and the restriction endonucleases DpnI and MboI were purchased from Boehringer Mannheim Biochemicals. Creatine phosphate (di-Tris salt) was obtained from Sigma. Deoxy- and ribonucleoside triphosphates were obtained from Yamasa Co. Radioactive materials were purchased from ICN Biochemicals Inc. Antipain was a gift from T. Aoyagi (Institute of Microbiological Chemistry, Tokyo, Japan). Plasmid pBE102 (18), which contains the PyV origin sequence, was provided by C. Prives (Columbia University). pBE102 DNA and pSV01ΔEP DNA (37) containing the SV40 origin sequence were prepared from dam methylase-positive Escherichia coli HB101 by the method of Maniatis et al. (21). Activated DNA was prepared with calf thymin DNA by the method of Aposhian and Kornberg (1). Single-stranded DNA–cellulose was prepared as described previously (32).

Cells and cell cultures. The cells used were FM3A cells (mouse mammary carcinoma cell line; Japanese Cancer Research Resource Bank [JCRB] 0701) (26), Ehrlich ascites tumor cells (mouse cell line; American Type Culture Collection [ATCC], CCL-77), V79 cells (Chinese hamster cell line; JCRB 0603), CV1 cells (African green monkey cell line; ATCC CCL-70), HeLa S3 cells (human carcinoma cell line; ATCC CCL-2.2), 293 cells (human cell line transformed by

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adenovirus DNA; ATCC CRL-1573), Spodoptera frugiperda 27 (S27) cells (insect cell line), and SJK287-38 cells (mouse hybridoma, anti-human DNA polymerase α antibody producer; ATCC CRL-1644). Ehrlich ascites tumor cells and V79 cells were obtained from the RIKEN Gene Bank (Tsukuba, Japan). HeLa S3 cells, 293 cells, and SJK287-38 hybridoma cells were obtained from ATCC. CV1 cells and S27 cells were provided by C. Prach and L. K. Miller (University of Georgia), respectively. S27 cells were maintained at 28°C in suspension cultures in modified Grace’s insect medium TC-100 (12) supplemented with 10% heat-inactivated fetal bovine serum.

Assays of DNA polymerase α and DNA primase activities. The standard reaction mixture for the DNA polymerase α activity assay (50 µl) consisted of 50 mM Tris-hydrochloride (pH 7.8); 50 mM KCl; 5 mM MgCl2; 1 mM dithiothreitol (DTT); 100 µM each dATP, dCTP, and dGTP; 50 µM [3H]dTP (74 GBq/mmol); 20% glycerol (vol/vol); and 250 µg of activated calf thymus DNA per ml. Reactions were carried out at 35°C for 30 min and processed as described previously (8).

DNA primase activity was assayed on the basis of the synthesis of RNA primers, reflected in the synthesized primer-coupled elongation of DNA on a poly(dT)350 template in the presence of the E. coli DNA polymerase I large fragment. The standard reaction mixture (30 µl) consisted of 20 mM Tris-hydrochloride (pH 8.0), 3.3 mM 2-mercaptoethanol, 0.2 mg of bovine serum albumin (BSA) per ml, 2 mM ATP, 2 mM MnCl2, 100 µM [3H]dATP (3.7 GBq/mmol), 42 µg of poly(dT)350 per ml, and 0.4 U of the E. coli DNA polymerase I large fragment. Reactions were carried out for 30 min at 35°C, and the radioactivity incorporated into acid-insoluble materials was measured as described previously (9).

One unit of activity was defined as the amount that catalyzed the incorporation of 1 nmol of dTMP and 1 nmol of dAMP into acid-insoluble materials during a 30-min incubation at 35°C for DNA polymerase α activity and DNA primase activity, respectively.

Preparation of cell extracts. FM3A, Ehrlich ascites tumor, HeLa S3, and S27 cells exponentially growing in suspension were harvested and washed twice with ice-cold calcium- and magnesium-free phosphate-buffered saline by centrifugation. In the case of adherent cells, CV1, V79, and 293 cells were washed twice with calcium- and magnesium-free phosphate-buffered saline containing 0.03% EDTA, soaked in the same buffer at room temperature for 15 min, and harvested by gentle pipetting and centrifugation. The precipitated cells were suspended in an equal volume of hypotonic buffer (20 mM potassium N-2-hydroxyethylpiperazin-N’-2-ethanesulfonic acid [HEPES] [pH 7.5], 5 mM KCl, 1.5 mM MgCl2, 1 mM DTT, 0.25 mM phenylmethylsulfonyl fluoride [PMSF], 2.5 µg of antipain per ml) and centrifuged at 1,500 rpm for 5 min. The cells were suspended in an equal volume of hypotonic buffer, kept at 0°C for 10 min, and homogenized in a Potter-Elvehjem-type Teflon-glass homogenizer. After the addition of 5 M NaCl to the homogenate (final concentration, 0.2 M), centrifugation was performed at 20,000 rpm for 20 min in a Beckman TLA 100.2 rotor at 2°C. The supernatant was dialyzed against buffer A (20 mM potassium HEPES [pH 7.0], 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.25 mM PMSF, 2 µg of antipain per ml, 10% glycerol) and centrifuged at 55,000 rpm for 15 min in the same rotor. The supernatant, containing 10 to 20 mg of protein per ml, was stored at −80°C until use.

Preparation of PyV T antigen. PyV T antigen was purified as described previously (28) from S27 cells infected with a recombinant baculovirus (Autographa california nuclear polyhedrosis virus) vector, vEV51LT (a gift from L. K. Miller) which expresses PyV T antigen.

Assay of PyV DNA replication. The reaction mixture (50 µl) for the PyV DNA replication assay consisted of 100 mM Tris-hydrochloride (pH 7.9); 7 mM MgCl2; 0.5 mM DTT; 3.5 mM ATP; 200 µM each dCTP, dGTP, and dUTP; 100 µM each dATP, dCTP, and dGTP; 25 µM [3H]dTTP (74 GBq/mmol); 40 mM creatine phosphate; 1 µg of phosphocreatine kinase; 0.2 to 0.4 µg of supercoiled duplex plasmid DNA containing the PyV DNA replication origin (pBE102, 6.2 kb); 1.5 to 2.0 µg of purified PyV T antigen; and various amounts of cell extracts. The reaction was performed for 90 min at 35°C and terminated by the addition of 0.2 ml each of 0.5 M sodium Pi and 2 mg of salmon sperm DNA per ml. After the addition of 5% trichloroacetic acid, acid-insoluble materials were collected on a Whatman GF/C glass filter. The radioactivity was measured with a liquid scintillation counter. PyV T antigen-dependent incorporation was regarded as replicative synthesis of PyV DNA in the cell-free system.

Assay of SV40 DNA replication. SV40 T antigen was purified from COS-1 cells infected with SV40 strain cs1085 by immunofinity chromatography with a monoclonal antibody against SV40 T antigen, Fab 419 (15), as described previously (37). The reaction mixture was essentially the same as that used for the PyV DNA replication assay, except that 0.3 µg of pSV01AEP DNA and 0.8 µg of purified SV40 T antigen were added in place of pBE102 DNA and purified PyV T antigen.

Product analysis. The reaction mixture (60 µl) for the product analysis contained all the components used for the assay of PyV DNA replication activity, except that 20 µM [α-32P]dCTP (185 GBq/mmol) and 100 µM dTTP were added in place of 100 µM dCTP and 25 µM [3H]dTTP. Reactions were carried out at 35°C for 90 min and terminated by the addition of 1 µl of 0.5 M EDTA and 3 µl each of 10% sodium dodecyl sulfate and 10 µg/ml trNA. The products were treated with 1 mg of proteinase K per ml for 30 min at 37°C. After extraction with phenol-chloroform (1:1), the products were precipitated with ethanol in the presence of 0.5 M ammonium acetate. The precipitate was dissolved in TE buffer (10 mM Tris-hydrochloride [pH 8.0], 1 mM EDTA) and electrophoresed in neutral agarose gels in Tris-acetate buffer (21). Gels were dried and subjected to autoradiography. For treatment of products with restriction endonucleases, products dissolved in TE buffer were precipitated again with ethanol, washed four times with ether, and dissolved in TE buffer. Digestion with DpnI and MboI was carried out in accordance with the supplier’s instructions, with the exception that the incubation with DpnI was carried out in the presence of 0.2 M NaCl; at lower concentrations, hemimethylated DNA and fully methylated DNA were cut by this enzyme (37).

Purification of DNA polymerase α-primase, isolated DNA polymerase α, and isolated DNA primase from mouse FM3A cells. FM3A cells were grown in mice and harvested as described previously (14). All operations described below were performed at 0 to 4°C. Cells (5 × 10^10) were washed with an equal volume of hypotonic buffer, suspended in an equal volume of hypotonic buffer, and kept at 0°C for 10 min. Cells were homogenized with 40 strokes in a Potter-Elvehjem-type Teflon-glass homogenizer, and 5 M NaCl was added to the homogenate (final concentration, 0.2 M) with gentle stirring. The homogenate was centrifuged at 40,000
rpm for 1 h in a Hitachi RP40 rotor, and the supernatant was dialyzed against solution A (0.1 mM EDTA, 0.01% [vol/vol] Triton X-100, 1 mM DTT, 0.25 mM PMSF, 2.5 μg of antipain per ml, 20% [vol/vol] ethylene glycol) containing 0.1 M potassium phosphate (pH 7.5). The dialysate was centrifuged to remove aggregates formed during dialysis and loaded onto a phosphocellulose column (4.5 cm [inner diameter] by 25 cm) equilibrated with the same solution. After the column was washed with solution A, proteins were eluted with solution A containing 0.3 M potassium phosphate (pH 7.5). The protein-rich fractions were pooled, mixed with an equal volume of solution A, and passed through a Sepharose CL-4B column (1.5 cm [inner diameter] by 10 cm). The pass-through fraction was loaded onto an anti-DNA polymerase α immunoaffinity column (1 cm [inner diameter] by 4 cm) which contained monoclonal antibody SJK287-38 cross-linked to Sepharose 4B and which was equilibrated with solution A containing 0.15 M potassium phosphate (pH 7.5). The column was washed with the same solution, and proteins bound to the column were eluted with solution A containing 20 mM potassium phosphate (pH 7.5) and 1 M KCl. A portion of the DNA primase activity was eluted by buffer A containing 1 M KCl. The fractions containing DNA primase activity were pooled, mixed with BSA (final concentration, 0.2 mg/ml), and concentrated with a small phosphocellulose column. The concentrated fractions were dialyzed against dialyzing buffer (20 mM potassium HEPES [pH 7.5], 0.1 mM EDTA, 50 mM NaCl, 1 mM DTT, 0.25 mM PMSF, 2.5 μg of antipain per ml, 50% glycerol). The dialysate was stored at −20°C as isolated DNA primase. DNA polymerase α-primase was eluted from the column with 50 mM diethanolamine-hydrochloride (pH 10.2)−1 M KCl-0.1 mM EDTA-1 mM DTT-0.01% Triton X-100-0.25 mM PMSF-2.5 μg of antipain per ml-30% ethylene glycol. After neutralization by the addition of 0.1 volume of 1 M potassium phosphate (pH 6.0), active fractions were pooled and dialyzed against dialyzing buffer. The dialysate was stored at −20°C as DNA polymerase α-primase.

Isolated DNA polymerase α was prepared from purified DNA polymerase α-primase essentially by a previously described method (30). The dialysate containing DNA polymerase α-primase was mixed with an equal volume of buffer D (20 mM potassium HEPES [pH 7.5], 0.1 mM EDTA, 1 mM DTT, 0.25 mM PMSF, 2.5 μg of antipain per ml) containing 50 mM NaCl and 0.4 mg of BSA per ml and was loaded onto a single-stranded DNA-cellulose column (0.5 cm [inner diameter] by 4 cm) equilibrated with buffer D containing 50 mM NaCl, 0.2 mg of BSA per ml, and 50% ethylene glycol. Proteins were eluted with a linear gradient of NaCl from 50 to 400 mM in buffer D containing 0.2 mg of BSA per ml and 50% ethylene glycol. DNA polymerase and DNA primase activities were eluted from the column, being partially separated at 200 and 280 mM NaCl, respectively. Fractions containing DNA polymerase activity but not DNA primase activity were pooled and concentrated with a small phosphocellulose column. The concentrated fractions were dialyzed against dialyzing buffer. The dialysate was stored at −20°C as isolated DNA polymerase α.

The specific activities of these enzyme preparations were as follows: DNA polymerase α-primase, 9.0 U of DNA polymerase activity and 15.8 U of DNA primase activity per μg of protein; isolated DNA polymerase α, 2.1 U of DNA polymerase activity per μg of protein (DNA primase activity was not detectable); and isolated DNA primase, 1.4 U of DNA primase activity and 0.04 U of endogenous DNA polymerase activity per μg of protein. It must be noted that the primase activity assayed by the system described here is enhanced by DNA polymerase α because of the reason given previously (30). Thus, the same unit of primase activity of DNA polymerase α-primase and isolated DNA primase does not mean the same amount of DNA primase. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that purified DNA polymerase α-primase contained four major bands of 180, 68, 54, and 46 kDa, as shown in Fig. 1 (lane 2) and as described previously (31). The DNA polymerase α preparation contained two bands of 180 and 68 kDa (Fig. 1, lane 3), and the DNA primase preparation contained two bands of 54 and 46 kDa (Fig. 1, lane 4).

RESULTS

Species-specific synthesis of PyV DNA and SV40 DNA in cell-free DNA replication systems. The replication activities of PyV origin-containing DNA and SV40 origin-containing DNA were measured in the presence of various amounts of extracts prepared from the cells of various species: human (HeLa S3 cells and 293 cells), monkey (CV1 cells), mouse (FM3A cells and Ehrlich ascites tumor cells), Chinese hamster (V79 cells), and insect (SF27 cells). Extracts prepared from FM3A cells, Ehrlich ascites tumor cells, or V79 cells supported PyV DNA synthesis, but extracts prepared from HeLa cells, 293 cells, or SF27 cells did not support PyV DNA synthesis (Fig. 2A). On the contrary, extracts prepared from HeLa cells, 293 cells, and CV1 cells supported SV40 DNA synthesis. Almost no DNA synthesis was observed with extracts prepared from FM3A cells, Ehrlich ascites tumor cells, V79 cells, and SF27 cells (Fig. 2B).

Restoration of replication activity in the cell-free replication system for PyV DNA containing nonpermissive cell extracts with mouse DNA polymerase α-primase. It was previously reported that FM3A cell extracts catalyzed the incorporation
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FIG. 2. Species-specific DNA synthesis in cell-free DNA replication systems for PyV DNA and SV40 DNA. The replicating activities of PyV DNA (A) and SV40 DNA (B) were assayed as described in Materials and Methods in the presence of the indicated amounts of cell extracts from mouse FM3A cells (●), mouse Ehrlich ascites tumor cells (▲), hamster V79 cells (△), monkey CV1 cells (○), human HeLa S3 cells (■), human 293 cells (□), or insect Sf27 cells (×).

of deoxyribonucleotides into SV40 DNA in the presence of SV40 T antigen when supplemented with HeLa cell DNA polymerase α-primase (25) and that HeLa cell extracts supported DNA synthesis in the cell-free system for PyV DNA in the presence of mouse DNA polymerase α-primase and PyV T antigen (24). Thus, we examined the PyV DNA-synthesizing ability of various nonpermissive cell extracts by the addition of DNA polymerase α-primase purified from mouse FM3A cells.

As shown in Fig. 3, an increase in the amount of incorporation of deoxyribonucleotides was observed with extracts prepared from HeLa, 293, CV1, and Sf27 cells, depending on the amount of mouse DNA polymerase α-primase added to the extracts.

Figures 4 and 5 show the results of product analyses with restriction endonucleases DpnI and MboI, respectively. Both DNA strands of PyV origin-containing plasmid DNA were methylated at adenine residues in the GATC sequence because this DNA was prepared from a dam methylase-positive E. coli strain. This DNA was sensitive to digestion by DpnI and resistant to digestion by MboI, since DpnI cut the GATC sequence when adenine residues on both strands were methylated and MboI cut the GATC sequence only when both strands were devoid of methylated adenine. Thus, DNA products replicated semiconservatively once are resistant to digestion by DpnI and products replicated multiply are sensitive to digestion by MboI.

Considerable portions of DNA products synthesized with extracts from HeLa cells and Sf27 cells in the presence of purified FM3A cell DNA polymerase α-primase were resistant to digestion by DpnI, as was the case with DNA products synthesized with extracts from FM3A cells and V79 cells (Fig. 4). Products migrating slower than relaxed circular DNA were partially sensitive to DpnI digestion because they were replicative intermediates containing various amounts of nonreplicated DNA, as described previously (37). The small amounts of radioactive materials produced with extracts from Sf27 cells in the absence of mouse DNA polymerase α-primase were digested by DpnI, indicating that they were derived from repair synthesis.

The appearance of MboI-digested fragments (Fig. 5) indicated that some of the DNA molecules underwent multiple rounds of replication, even in reaction mixtures containing nonpermissive cell extracts prepared from HeLa cells and Sf27 cells, in the presence of mouse DNA polymerase α-primase.

Induction of PyV DNA replication in HeLa cell extracts by the addition of isolated mouse DNA primase. We next examined whether PyV DNA replication is restored in nonpermissive cell extracts by the addition of either isolated mouse DNA polymerase α or isolated DNA primase. The enzyme preparations purified from mouse FM3A cells contained two protein bands of 180 and 68 kDa (DNA polymerase α) and 54 and 46 kDa (DNA primase), as indicated in Fig. 1, lanes 3 and 4, respectively.

No increase in the amount of incorporation of deoxyribonucleotides was observed with the addition of isolated.
FIG. 4. Analysis of products synthesized in the cell-free replication system containing nonpermissive cell extracts supplemented with purified mouse DNA polymerase α-primase by digestion with DpnI. The reaction mixture contained 400 µg of the indicated extract and 2 µg of PyV T antigen. Reactions were carried out at 35°C for 90 min in the presence (+) or absence (−) of purified FM3A cell DNA polymerase α (polα)-primase (2.0 U, based on DNA polymerase α activity). DNA products were processed as described in Materials and Methods and incubated at 37°C for 90 min in the presence (+) or absence (−) of 10 U of DpnI. The incubated products were electrophoresed in a 0.5% agarose gel. The positions at which supercoiled DNA (RFI) and relaxed circular DNA (RFII) migrated were determined by ethidium bromide staining. The values for the incorporation of dCMP obtained with various cell extracts in the presence or absence of purified DNA polymerase α-primase were 69.4 (absence) pmol for FM3A cell extracts, 15.5 (absence) pmol for V79 cell extracts, 4.3 (absence) and 26.0 (presence) pmol for HeLa cell extracts, and 3.0 (absence) and 38.0 (presence) pmol for Sf27 cell extracts. The percentages of incorporated radioactivity resistant to DpnI digestion were 79.0 for FM3A cell extracts, 60.9 for V79 cell extracts, 3.0 (absence) and 82.0 (presence) for HeLa cell extracts, and 8 (absence) and 47.5 (presence) for Sf27 cell extracts.

FM3A cell DNA polymerase α to HeLa cell extracts (Fig. 6A) and Sf27 cell extracts (Fig. 6B). When FM3A cell DNA primase was added to HeLa cell extracts, the incorporation of deoxyribonucleotides increased, as was the case with the addition of DNA polymerase α-primase (Fig. 6A). Activation of DNA synthesis by the addition of FM3A cell DNA primase was also observed with 293 cell extracts and CV1 cell extracts (data not shown). In contrast, no activation of PyV DNA synthesis was observed with Sf27 cell extracts when FM3A cell DNA primase was added (Fig. 6B). Like DNA polymerase α-primase, the mixture of isolated DNA polymerase α and isolated DNA primase induced DNA synthesis in HeLa cell extracts and Sf27 cell extracts, although the level of induction by the mixture was lower than that by DNA polymerase α-primase.

The restoration of the incorporation of deoxyribonucleotides in HeLa cell extracts by the addition of isolated DNA primase was suggested to be due to DNA replication but not to repair synthesis, because approximately 24% of the products synthesized in this system were resistant to DpnI digestion (Fig. 7). No DpnI digestion-resistant materials were observed with Sf27 cell extracts when isolated DNA primase was added.

DISCUSSION

In this study, we have analyzed the species-specific replication of PyV DNA in a cell-free system containing PyV
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FIG. 7. Analysis of products synthesized in the cell-free replication system containing nonpermissive cell extracts supplemented with mouse DNA polymerase α-primase or DNA primase by digestion with DpnI. The reaction mixture contained cell extracts (250 µg of protein) from FM3A, HeLa, or SF27 cells and 1.5 µg of PyV T antigen. Reactions were carried out at 37°C for 90 min in the presence (+) or absence (−) of 1.2 U (based on primase activity) of FM3A cell DNA polymerase α (polo-primase) DNA primase. DNA products were treated as described in the legend to Fig. 4. The percentages of incorporated radioactivity resistant to DpnI digestion were 55.7 for FM3A cell extracts, 33.8 for HeLa cell extracts with the FM3A cell DNA polymerase α-primase complex, 23.6 for HeLa cell extracts with the FM3A cell DNA primase, 57.0 for SF27 cell extracts with the FM3A cell DNA polymerase α-primase complex, and 0 for SF27 cell extracts with the FM3A cell DNA primase.

origin-containing DNA, PyV T antigen, and extracts from cells of various species, and several important features have been revealed, as follows.

First, extracts prepared from two mouse cell lines, FM3A and Ehrlich ascites tumor, and a hamster cell line, V79, supported PyV DNA replication in the presence of PyV T antigen, but those prepared from human cell lines, HeLa and 293, and a monkey cell line, CV1, did not. On the contrary, extracts inactive for PyV DNA replication supported SV40 DNA replication, and extracts active for PyV DNA replication were not active for SV40 DNA replication (Fig. 2). Extracts prepared from an insect cell line, SF27, supported neither PyV DNA replication nor SV40 DNA replication. These results correlate with the host range specificities of both viruses, except for one instance, in which extracts prepared from hamster V79 cells, which are semipermissive for PyV and SV40 propagation (33), supported PyV DNA replication but not SV40 DNA replication.

Second, DNA polymerase α-primase isolated from a permissive cell line, FM3A, induced PyV DNA synthesis in a cell-free system containing nonpermissive extracts of human, monkey, or insect cells (Fig. 3). Analysis with restriction endonuclease DpnI indicated that DNA synthesis was induced by semiconservative replication rather than repair synthesis (Fig. 4). The appearance of MboI-digestible products indicated that multiple rounds of replication occurred in the cell-free system containing nonpermissive cell extracts when it was supplemented with mouse DNA polymerase α-primase. These results are consistent with earlier observations indicating that DNA polymerase α-primase plays a major role in determining host cell permissiveness for parovavirus DNA replication (24, 25, 36, 38), probably by interacting with T antigen (7, 28).

The levels of induced DNA synthesis, however, were much lower with nonpermissive cell extracts than with permissive cell extracts (Fig. 3). This result may imply the existence of some trans-acting factors (35) in nonpermissive cell extracts which inhibit the interaction between T antigen and DNA polymerase α-primase.

Gannon and Lane showed that mouse p53 competes with human DNA polymerase α for binding to the SV40 T antigen (11). Braithwaite et al. also found that the induction of mouse p53 resulted in the inhibition of SV40 origin-dependent DNA replication in monkey COS cells (3). Furthermore, Wang et al. reported that purified mouse p53 inhibited SV40 DNA replication in a cell-free system, blocking the initiation stages of DNA replication (34). Thus the host range specificity of SV40 may be partially explained by the inhibitory effect of p53, although p53 isolated from COS cells that are permissive for SV40 recently has been shown to inhibit SV40 DNA replication in the cell-free system (33). However, PyV T antigen does not seem to associate with p53 (34). Thus, the low level of induced PyV DNA replication is not likely to be due to the existence of p53 but rather to the existence of other inhibitory factors or to the absence of the factors in permissive cells which may participate in host range specificity.

Third, like mouse DNA polymerase α-primase, mouse DNA primase was able to induce PyV DNA replication in human cell extracts (Fig. 6 and 7). This fact indicates that DNA primase but not DNA polymerase α plays the principal role in the determination of permissiveness for PyV DNA replication and also that human DNA polymerase α supports PyV DNA replication in concert with mouse DNA primase.

The inability of mouse primase to activate insect cell extracts may be explained by the possibility that mouse primase is not able to interact with insect DNA polymerase α. The discrepancy between the results reported here and the results reported previously that SV40 DNA replication was restored in mouse cell extracts only when both HeLa cell DNA primase and DNA polymerase α were added to mouse cell extracts also may be explained by the similar possibility that HeLa cell DNA primase is not able to interact with mouse DNA polymerase α.

The association of DNA primase with DNA helicase has been observed in several systems. The T4 phage gene 61 protein, which is a primase, forms a complex with DNA helicase, the gene 41 protein (4). The gene products of UL52, UL5, and UL8 of herpes simplex virus type 1 form a ternary complex having both DNA primase and helicase activities (5). Recently, we have shown that PyV T antigen, like SV40 T antigen, has DNA helicase activity (27). Furthermore, we have observed that PyV T antigen facilitates RNA primer synthesis catalyzed by isolated mouse DNA primase on a single-stranded DNA template (data not shown). Thus, it seems likely that mouse DNA primase directly interacts with PyV T antigen.

However, Dornreiter et al. (7), using an immunochemical method, indicated that SV40 T antigen interacted directly with the large subunit of DNA polymerase α but not with either of the primase subunits. The reason why SV40 T antigen did not interact with DNA primase is not clear at present. The following observation seems to indicate that...
HeLa cell DNA primase interacts with SV40 T antigen indirectly via DNA polymerase α. DNA primase-catalyzed RNA primer synthesis in the reconstituted SV40 DNA replication system required DNA polymerase α in addition to SV40 T antigen and single-stranded DNA binding protein (22).

In conclusion, mouse DNA primase plays the principle role in the determination of permissiveness for PyV DNA replication, probably by interacting with PyV T antigen.

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