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Chinese Hamster Ovary Cells Replicate Adenovirus Deoxyribonucleic Acid

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Chinese hamster ovary (CHO) cells infected with adenovirus type 2 (Ad2) produced amounts of viral deoxyribonucleic acid (DNA) equal to that synthesized in permissively infected HeLa cells. However, there was 6,000-fold less virion produced in CHO cells. Since the structural viral polypeptides were not detected by pulse-labeling CHO cells at various times postinfection, the block in virion formation is located between the synthesis of viral DNA and late proteins. Extracts of CHO cells could also function in a recently reported in vitro Ad2 DNA synthesis system which is dependent upon the addition of exogenous Ad2 DNA covalently linked to a 5'-terminal protein (Ikeda et al., Proc. Natl. Acad. Sci. U.S.A. 77:5827-5831, 1980). Extracts of infected CHO cytoplasm were able to complement uninfected CHO nuclear extracts to synthesize viral DNA on Ad2 templates. This in vitro replication system has the potential to probe host DNA synthesis requirements as well as viral factors.

The ability to obtain mutations in many of the proteins involved in procaryotic deoxyribonucleic acid (DNA) synthesis has led to the elucidation of the mechanism by which bacterial and bacteriophage DNAs are replicated (6, 17, 23, 29). The difficulty in selecting appropriate eucaryotic mutants has limited the advance in our understanding of eucaryotic DNA synthesis. However, considerable success has been achieved recently in the selection of mutants in a variety of biochemical pathways in mammalian somatic cells (1, 26, 27). Some of the most significant advances in selecting recessive mutations have been in the Chinese hamster ovary (CHO) cell. At the outset of our studies to obtain conditionally lethal CHO mutants in DNA synthesis, this cell line was considered to be a nonpermissive host for adenovirus (Ad) replication. CHO cells do produce 6,000-fold less infectious virion than a permissive cell such as the HeLa S3 line. However, the data in this report will demonstrate that CHO cells can be infected with Ad type 2 (Ad2) and support amounts of viral DNA synthesis equivalent to that synthesized during a productive infection. Ad DNA synthesis in CHO cell-free extracts correlates well with the studies of viral DNA synthesis in cell cultures. Extracts from the cytoplasm of Ad2-infected CHO cells, added to extracts of uninfected CHO nuclei, synthesize full-length viral DNA upon addition of an Ad2 DNA template covalently linked to a 5'-terminal protein (Ad2 DNA-PRO).

With the increasing number of conditionally lethal DNA synthesis mutants recognized in somatic cells, it may be possible to use the in vitro Ad DNA synthesis system to determine how a particular cell mutation affects DNA replication.

(The data in this paper were submitted by M.L. as part of a thesis for the Ph.D. degree from the Sue Golding Graduate Division of Medical Sciences, Albert Einstein College of Medicine, Bronx, N.Y.)

Cells and virus. The sources of HeLa S3 cells and Ad2 have been previously described (20). The cells were grown in suspension culture in Eagle minimum essential medium supplemented with 5% fetal calf serum and 1% glutamine. CHO cell suspensions were obtained from Pamela Stanley of the Albert Einstein College of Medicine and were grown on alpha medium (GIBCO) with 5% fetal calf serum but without added ribo- and deoxyribonucleosides. The CHO cell line is a proline auxotroph and is designated $Pro^{-}5$. Cell cultures were infected by adding purified Ad2 (4,000 virions per cell) as previously described (18).

Characterization of virion, viral DNA, and proteins. The techniques of radioactive labeling, alkaline sucrose gradient centrifugation, and DNA-DNA hybridization have been previously described (8). Virus was purified and quantitated on CsCl gradients (10). Polypeptides were characterized on 12.5% polyacrylamide vertical slab gels containing sodium dodecyl sulfate (19).

Preparation of uninfected NE and Ad2-infected cytosols. Uninfected nuclear extracts (NE) were prepared from cells which were washed and processed by Dounce homogenization according to the procedure of Challberg and Kelly for infected cells (4); however, hydroxy-

urea (HU) was not added to uninfected cultures. Nuclei were rapidly frozen in liquid nitrogen and later stored at −70°C. Frozen nuclei were thawed in ice water and extracted with 100 mM NaCl for 10 min at 0°C. The extracted nuclei and nuclear debris were removed by centrifugation for 20 min at 15,000 × *g* in a Beckman type 40 rotor.

Ad2-infected cytosol was processed after separation from infected nuclei prepared by Dounce homogenization. For Ad2-infected HeLa cells, 3 mM HU was added 2 h postinfection (p.i.), and cells were harvested 21 h p.i. When CHO cells were infected with Ad2, 0.4 mM HU was added 2 h p.i., and Ad2-infected cytosols were prepared at 46 h p.i. Unfractionated cytoplasms were stored at −70°C. After thawing, either HeLa or CHO infected cytosols were centrifuged for 30 min at 100,000 × *g* in a Beckman type 40 rotor. The clarified supernatant was partially purified and concentrated by a 25 to 60% ammonium sulfate precipitation (11). The precipitate was dissolved in TMEG [25 mM tris(hydroxymethyl)aminomethane-hydrochloride, pH 7.4, 1 mM ethylenediaminetetraacetic acid, 1.5 mM β-mercaptoethanol, and 10% glycerol) plus 50 mM NaCl and dialyzed twice against 200 volumes of the same buffer. The 25 to 60% precipitate from the cytosol of 1 liter of infected cells (5×10^5 cells per ml) was suspended in 1 ml of TMEG buffer with 50 mM NaCl. Ad2-infected cytosols prepared from HeLa cells contained 17.5 mg of protein per ml; those from CHO cells contained 13.5 mg/ml.

Preparation of radioactively labeled Ad2 DNA and DNA-PRO. [^{14}C]thymidine-labeled Ad2 virion and purified ^{14}C-labeled deproteinized Ad2 DNA were prepared as described (8). Ad2 [^{14}C]DNA-PRO was purified from virions by the method of Sharp et al. (25). The peak fractions of Ad2 DNA-PRO from guanidine-containing sucrose gradients were dialyzed against four changes of 100 volumes of 10 mM tris(hydroxymethyl)aminomethane (pH 7.4) and 1 mM ethylenediaminetetraacetic acid before storage at −70°C.

Synthesis of DNA in vitro. Various combinations of uninfected NE and the 25 to 60% ammonium sulfate fraction from infected cytosol were incubated for 120 min at 37°C in reaction mixtures (0.10 ml) containing 50 mM HEPES (*N*-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (pH 7.5), 0.5 mM dithiothreitol, 5 mM $MgCl_2$, 3.8 mM adenosine 5′-triphosphate, 50 μM each of deoxyadenosine 5′-triphosphate, deoxycytidine 5′-triphosphate, and deoxyguanosine 5′-triphosphate, 6 μM deoxy[^{3}H]thymidine 5′-triphosphate (2.5×10^3 cpm/pmol), and 0.15 μg of Ad DNA or DNA-PRO template. After incubation, acid-insoluble radioactivity was quantitated as described (14).

DNA synthesis using *Xba*I restriction endonuclease fragments of Ad2 DNA-PRO. Ad DNA-PRO (0.15 μg) was digested with 3.5 U of *Xba*I in 20 μl of 20 mM HEPES (pH 7.5), 10 mM NaCl, and 7 mM $MgCl_2$ for 60 min at 37°C. After digestion with restriction endonuclease, the cleaved DNA-PRO was added to a reaction mixture (100 μl) as described above except that 1.5 μM α-deoxythymidine 5′-[^{32}P]triphosphate replaced the deoxy[^{3}H]thymidine 5′-triphosphate. After addition of uninfected NE or Ad2 cytosol fraction or both, the mixture was incubated for 60 min at 37°C, and the reaction was terminated with 0.2% sodium dodecyl sulfate. Pronase (100 μg/ml, 37°C, 20 min) was added to remove covalently linked terminal protein, and the DNA was precipitated for 16 h with 2.5 volumes of ethanol in the presence of 0.5 M sodium chloride. The DNA was solubilized in 50 μl of TEA buffer [40 mM tris(hydroxymethyl)-aminomethane, pH 7.8, 1 mM ethylenediaminetetraacetic acid, 5 mM sodium acetate] and electrophoresed on 1.4% agarose slab gels containing the same buffer until the bromophenol blue dye reached the lower edge of the gel. The gels were stained with ethidium bromide (0.5 μg/ml), photographed, dried, and autoradiographed for 18 h.

Materials. Radioisotopes were purchased from Schwarz-Mann and New England Nuclear Corp. The *Xba*I restriction endonuclease was obtained from Bethesda Research Laboratory.

RESULTS

Rate of synthesis of viral DNA in CHO cells. After infection of CHO suspension cultures with Ad2, the rate of viral DNA synthesis was measured by pulse-labeling infected cells with [^{3}H]thymidine and separating viral from host DNA on alkaline sucrose gradients. Viral DNA synthesis was detectable on gradients at 18 h p.i. but was not present at 12 h p.i. (Fig. 1). The rate of viral DNA synthesis was maximal at 36 h p.i. The identity of the viral peak was confirmed by DNA-DNA hybridization (data not shown).

Total viral DNA synthesized in CHO and HeLa cells. Suspension cultures of CHO and HeLa cells were infected with purified Ad2. At the time of infection and every 12 h p.i., 2.5 μCi of [^{14}C]thymidine was added to each culture. At 40, 60, and 84 h p.i., 5-ml samples from each infection were removed, the cells were sedimented on alkaline sucrose gradients, and the cumulative amount of viral DNA synthesized in each cell type was determined (Fig. 2). In addition, at 84 h p.i., the optical density was moni-

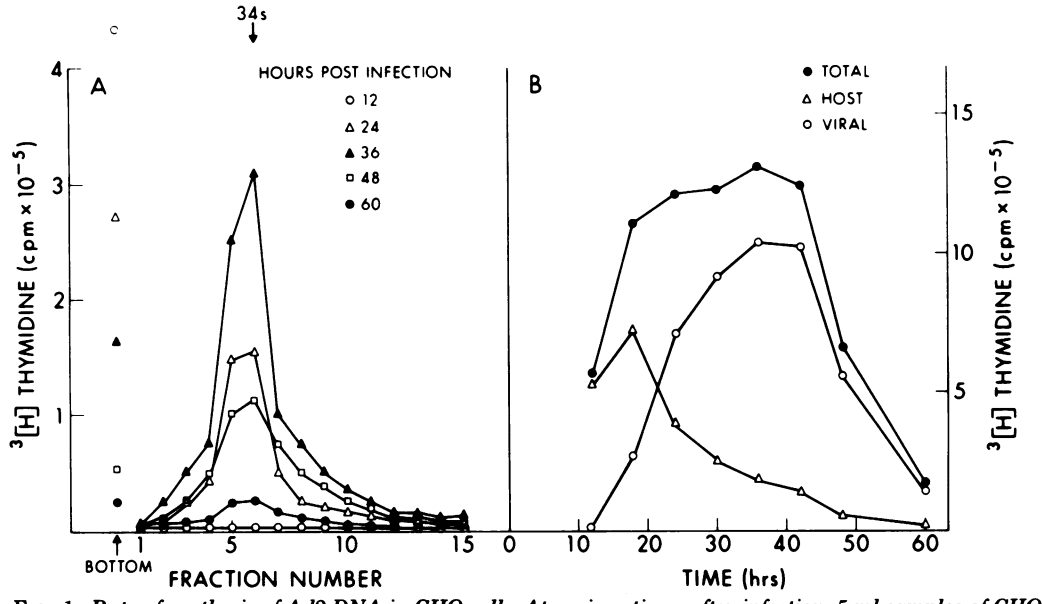

FIG. 1. *Rate of synthesis of Ad2 DNA in CHO cells. At various times after infection, 5-ml samples of CHO cells (4 × 10^5 cells per ml) were labeled with 25 μCi of [^{3}H]thymidine for 1 h. After the labeling period, the cells were washed free of radioactivity, suspended in a medium, and chased with 2 × 10^{-5} M thymidine for an additional 30 min to complete the elongation of any partially replicated radioactive molecules. After the chase, the cells were centrifuged, suspended in 0.5 ml of sodium chloride (0.15 M), and placed intact onto 5 to 20% alkaline sucrose gradients for centrifugation at 24,000 rpm for 16 h in a Beckman SW27.1 rotor. Gradients from five of the eight time points appear in (A). The bottom of the gradient where the host DNA sedimented is designated. The arrow marks the 34S position on the gradient where marker Ad DNA was found. Sedimentation is from right to left. (B) Amount of viral, host, and total DNA synthesized during each 1-h pulse. The numbers were calculated from the radioactivity in the host and viral regions of the gradients in (A). The recovery of counts from each gradient was approximately 90%.*

tored during the collection of fractions from the gradients. A similar amount of DNA was synthesized in both HeLa and CHO cells as shown by absorbance at 260 nm as well as incorporation of radioactive thymidine.

Ad2 virion production in CHO and HeLa cells. The amount of Ad2 virion produced by infected CHO and HeLa cells was determined at 84 h p.i. by purification of virus on CsCl gradients (Fig. 3) and by infectivity assay. Although a radioactive virus peak was easily seen in HeLa cell extracts, there was no virus detected by radioactive labeling of CHO cells.

The infectivity titers of both CHO and HeLa cell lysates were determined by serial 10-fold dilutions on HeLa cell monolayers. After 10 days of incubation, the endpoints of the titration were determined. The tissue culture infectivity dose of virion produced in HeLa cells (2 × 10^3 50% tissue culture infective doses per cell) was greater than 6,000-fold the amount of virion produced in CHO cells (0.3 50% tissue culture infective dose per cell).

Ad DNA synthesis in vitro catalyzed by uninfected NE and infected cytosol. Uninfected NE and Ad2-infected cytosols were prepared from both HeLa cells and CHO cells as described in Materials and Methods. When preparing CHO Ad2 cytosol, 0.4 mM HU was used instead of 3 mM HU as routinely used for HeLa cells. The decrease in HU concentration was needed because of its toxicity at 3 mM during the longer incubation time for the CHO cells (46 h) as compared to that for HeLa cells (21 h). However, at a concentration of 0.4 mM HU, greater than 90% inhibition of viral DNA synthesis could still be obtained. Uninfected CHO NE and Ad2-infected CHO cytoplasm were incubated individually with Ad DNA-PRO. The DNA synthesized was mostly small and near the top of the gradients (Fig. 4A). However, when these two extracts were combined, the newly synthesized Ad DNA sedimented as full-sized molecules. In addition, uninfected NE from either CHO or HeLa cells could complement Ad2-infected cytosol from HeLa cells (Fig. 4B).

A recently developed assay for testing the specificity of in vitro DNA replication was also used to analyze the products (M. S. Horwitz and H. Ariga, Proc. Natl. Acad. Sci., in press). The

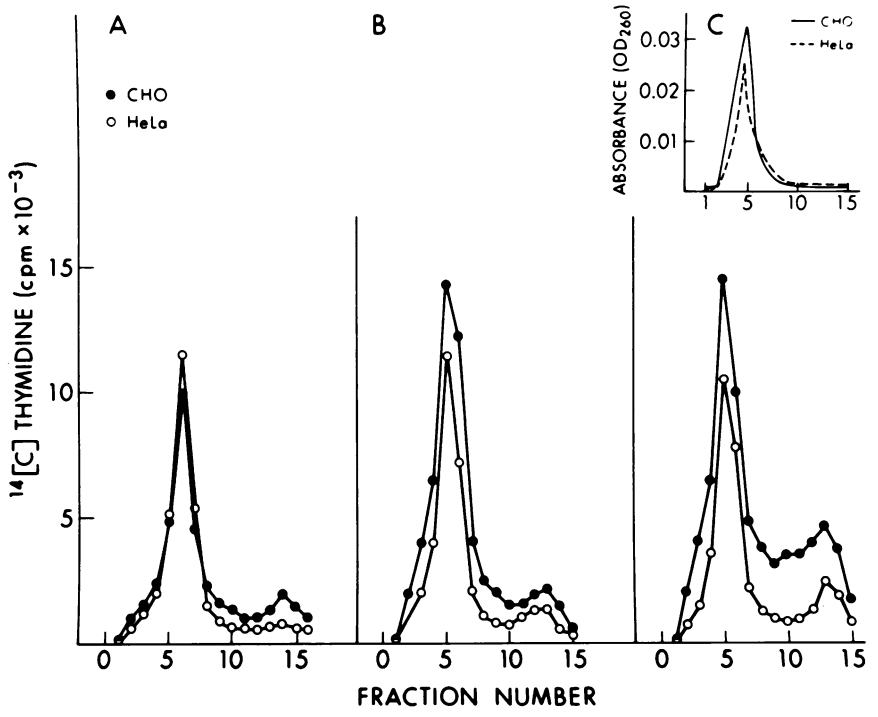

FIG. 2. *Accumulation of viral DNA in CHO and HeLa cells. CHO and HeLa cells (3×10^5 cells per ml; 300 ml of each) were infected with Ad2. [^{14}C]thymidine (2.5 μCi) was added to each culture at the time of infection and at 12-h intervals p.i. At various times, 5-ml samples of cells from both cultures were centrifuged and layered onto alkaline sucrose gradients as described for Fig. 1A. Viral DNA synthesized (A) from 0 to 40 h p.i.; (B) from 0 to 60 h p.i.; and (C) from 0 to 84 h p.i. The insert in (C) shows the absorbance (260 nm) of the fractions collected from the gradients at 84 h p.i., with the background absorbance from uninfected cells subtracted. The host DNA counts in the pellets were 9,250 cpm for HeLa and 33,100 cpm for CHO at 40 h p.i.; 7,290 cpm for HeLa and 20,400 cpm for CHO at 60 h p.i.; and 8,119 cpm for HeLa and 39,431 cpm for CHO at 84 h p.i.*

assay depends on the specific labeling of the Ad2 DNA-PRO at both ends of the molecule where replication has been shown to initiate (reviewed by Winnacker [31]). Ad DNA-PRO was digested for 1 h with the restriction endonuclease *Xba*I, and the resulting fragments without any further purification were added to a DNA synthesis reaction containing various combinations of HeLa and CHO infected cytosols and uninfected NE (Fig. 5). After 1 h of DNA synthesis, the viral DNA fragments were deproteinized, precipitated with ethanol to remove the radioactive deoxythymidine 5'-triphosphate, and electrophoresed in an agarose gel (Materials and Methods). The results indicate that the terminal DNA fragments C and E were primarily labeled by the in vitro DNA synthesis reaction when both components of the reaction were present (Fig. 5). If synthesis was attempted on the Ad DNA-PRO by using uninfected NE alone, there was considerable nonspecific incorporation into all of the restriction endonuclease fragments. The synthesis with Ad DNA-PRO plus infected cytosol alone was minimal, but the radioactivity did concentrate in the terminal fragments. It can be seen that the addition of infected cytosol to uninfected NE considerably reduced the nonspecific incorporation of radioactivity by the NE alone. If the terminal protein was removed from the DNA before the synthesis reaction, there was only a small amount of radioactive incorporation, which was distributed simply according to the size of the fragments and therefore appeared primarily in the large fragments A and B (Horwitz and Ariga, in press). Thus the results of the gel assay using CHO uninfected NE and infected cytoplasms, as well as those from analysis of alkaline sucrose gradients (Fig. 4), indicate that the extracts of CHO infected cells can substitute specifically for HeLa cell fractions.

Analysis of late viral proteins in infected CHO and HeLa cells. Although Ad DNA can be normally synthesized in CHO cells, the synthesis of "late" Ad proteins is severely reduced (Fig. 6). None of the viral structural proteins was discernible at 36 h p.i. in infected CHO lysates.

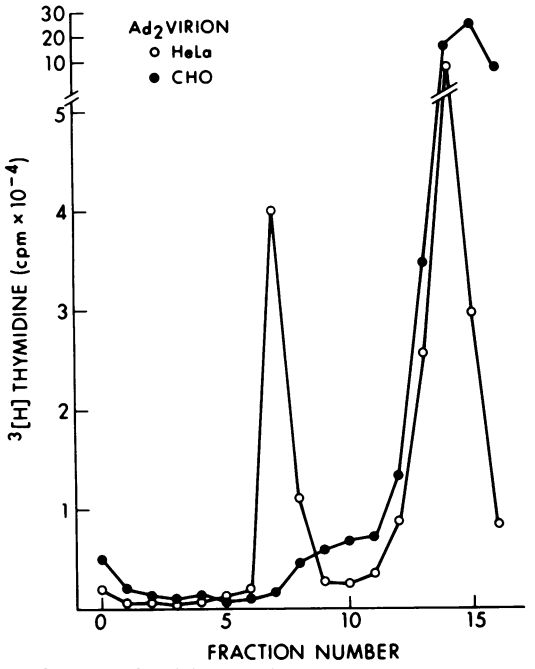

FIG. 3. *Ad2 virion production in CHO or HeLa cells. Ad2-infected HeLa or CHO cells (3 × 10^5 cells per ml; 50-ml samples) labeled with [^{14}C]thymidine in the same experiment as shown in Fig. 2 were removed at 84 h p.i. Virions from infected cells were partially purified as previously described (10). After sonication of the concentrated cells (1 ml in 0.01 M Tris, pH 8.1), addition of deoxycholate (0.5%), and freon extraction, the aqueous extract was centrifuged on 15.5-ml preformed linear CsCl gradients (ρ = 1.2 to 1.4g/ml) for 3 h at 24,000 rpm in a Beckman SW27.1 rotor. The sedimentation is from right to left, and the virion appears in fraction 7.*

However, there was a band of approximately 75,000 daltons that was not present in uninfected CHO cells and may be the Ad2 DNA-binding protein. Similar experiments with continuous labeling of infected CHO cells from 24 to 72 h p.i. did not reveal any viral structural polypeptides (data not shown). In contrast, viral polypeptides were synthesized in Ad2-infected HeLa cells at 24 h p.i. (Fig. 6). Therefore, it appears that the block to Ad virion production can be located between the synthesis of viral DNA and that of structural proteins.

DISCUSSION

In CHO cells, the onset of Ad2 DNA replication and the corresponding inhibition of host DNA synthesis is later than comparable infection of HeLa cells. In HeLa cells, viral DNA synthesis begins approximately 8 to 9 h p.i. and reaches a peak at 16 to 18 h p.i. (8). The inhibition of cellular DNA synthesis in these cells begins at 4 to 6 h p.i. and reaches 90% inhibition by 16 h p.i. (8). In CHO cells, there was no Ad2 DNA detected at 12 h either by gradient analysis (Fig. 1) or by DNA-DNA hybridization on Millipore HA filters; in these cells, the rate of Ad2 viral DNA synthesis was maximal at 36 to 40 h after infection. Cellular DNA synthesis was inhibited 90% by 40 h p.i. in CHO cells. However, when the cumulative amounts of Ad2 DNA synthesized in CHO and HeLa cells are compared (Fig. 2) it is apparent that similar amounts of viral DNA were produced.

Although the yield of Ad2 DNA was normal in CHO cells, only small amounts of infectious virions were found. In these cells, no virion could be detected by density gradient analysis up to 84 h p.i. (Fig. 3). Titration of infectivity indicated that the amount of virion produced in CHO cells is at least 6,000-fold lower than in HeLa cells. Approximately 0.3 infectious particles were detected per infected CHO cell although the input multiplicity of infection was approximately 80 plaque-forming units per cell, representing 4 × 10^3 particles per cell. Some of the apparent CHO progeny virions may actually be input infectious particles which have escaped the eclipse phase.

None of the Ad structural polypeptides was detected by pulse-labeling infected CHO cells at various late times in the infectious cycle. It is presumed that the early viral coded proteins, including the 72,000-molecular weight (72K) Ad2 DNA binding protein necessary for viral DNA synthesis (9, 13, 30), are synthesized normally, and a band that is approximately 75,000 daltons did appear to be labeled after infection (Fig. 6). These data would place the block in virion formation between the synthesis of viral DNA and that of structural polypeptides. Previous studies with Ad2 infection of another species of hamster cells, baby hamster kidney (BHK-21) cells (5), showed kinetics of DNA synthesis similar to the results obtained with CHO cells. However, in contrast to the present studies with CHO cells, relatively normal amounts of infectious virion were produced in BHK-21 cells.

The block of virion formation in CHO cells is more similar to that of the extensively studied Ad growth defect in monkey cells (21). In the latter system, DNA synthesis is also approximately normal (21, 22), but there is a defect in structural polypeptide synthesis (2, 3, 24). The inhibition of viral protein synthesis in CHO cells seems more extensive, however, than the inhibition of fiber and other capsid proteins in the monkey system (2, 7, 16).

Our experiments in CHO cells were initiated with the aim of studying Ad DNA replication in

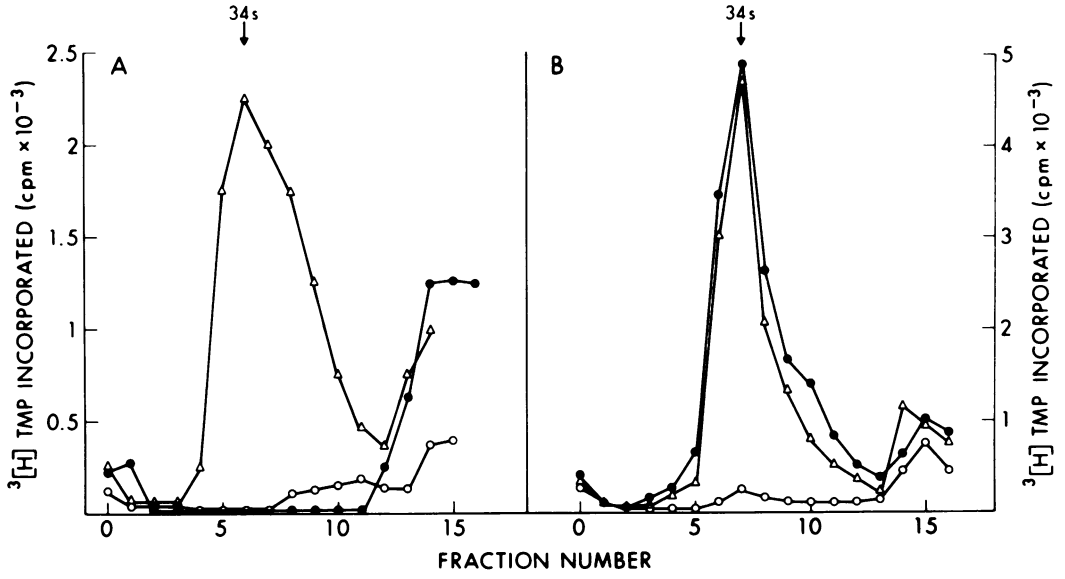

FIG. 4. *In vitro synthesis of Ad2 DNA by various mixtures of CHO or HeLa infected cytosols and uninfected NE. All reaction mixtures contained Ad2 DNA-PRO (0.15 μg) and were incubated at 37°C for 120 min as described in the text. Reaction mixtures (100 μl) with NE contained 20 μl of either CHO NE (52 μg of protein) or HeLa NE (54 μg). Those with cytosol contained either 10 μl of Ad2-infected CHO cytosol (135 μg) or 5 μl of Ad-infected HeLa cytosol (88 μg). The reactions were terminated by the addition of 0.3 ml of 0.1 M ethylenediaminetetraacetic acid–0.15 M NaCl at 0°C. After removal of 0.08 ml for quantitation of deoxythymidine 5'-monophosphate incorporation, the remaining 0.32 ml was layered onto 5 to 20% alkaline sucrose gradients and centrifuged at 24,000 rpm in a Beckman SW27.1 rotor for 16 h at 4°C. The direction of sedimentation in these gradients is from right to left. The arrow represents the position of the 34S marker Ad [^{14}C]DNA single strands. Symbols and amounts incorporated are: (A) CHO infected cytosol (○; 1.0 pmol), CHO NE (●, 1.8 pmol), CHO NE + CHO infected cytosol (△, 6.2 pmol); (B) HeLa infected cytosol (○, 1.4 pmol), HeLa infected cytosol + CHO NE (●, 11.8 pmol), HeLa infected cytosol + HeLa NE (△, 8.7 pmol).*

cells which might be optimal for obtaining conditionally lethal DNA synthesis mutants. CHO cells which have been extensively studied and mutagenized in many cell functions could serve as a source of mutants in DNA synthesis. We therefore tested extracts from CHO cells in place of HeLa cell extracts for their ability to support Ad DNA synthesis in vitro. The in vitro system developed in our laboratory is a modification of the infected NE reported by Challberg and Kelly (4). We have been able to replace their infected NE with two separate components: an uninfected cell NE and a fraction from Ad-infected cytoplasm (11; Horwitz and Ariga, in press).

Our results demonstrate that extracts of uninfected CHO nuclei or infected CHO cytoplasm can readily substitute for corresponding HeLa cell fractions. The size distribution of single-strand DNA synthesized in CHO cells is broader than in HeLa cells (Fig. 4). The smaller molecules are probably replicating intermediates generated because infected CHO cytosol fractions were 50% as active as similar fractions from HeLa cells. This corresponds to reduced rates but normal yields of DNA in cultures of CHO cells (Fig. 1 and 2). The results of the agarose gel fragment assay (Fig. 5) using these same extracts indicated that repair-type synthesis is not the cause of the broadened peak.

The biochemical goal of purifying the DNA replication system into component enzymatic activities is being actively pursued in both NE and infected cytoplasmic extracts. Since Ad is not known to code for any new DNA polymerases (12), these essential enzymes are probably present in our uninfected cell extracts. We have found that DNA polymerase β purified from HeLa cells can replace most of the nuclear requirement for in vitro Ad DNA synthesis (11). The synthesis with DNA polymerase β is 70 to 80% of the level obtained with uninfected NE, and the viral DNA product synthesized with either of these reagents is full length. We and others have shown that the combination of DNA polymerases α plus β can extensively replicate short primers that are hydrogen-bonded to φX single-strand virion DNA (11, 15, 28). There is a considerable amount of DNA polymerase α in our cytoplasmic preparation so that further enhancement of cytoplasmic activity on Ad DNA-

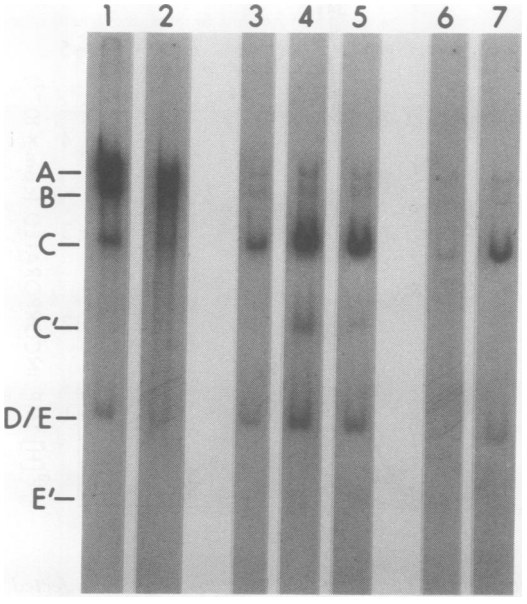

FIG. 5. *DNA synthesis on restriction endonuclease fragments of Ad2 DNA-PRO. Ad DNA-Pro (0.15 μg) was digested with XbaI, and the DNA fragments were added to the reaction mixtures with various combinations of Ad2 cytoplasm and uninfected NEs (see the text). The radioactive DNA products of the synthesis reaction are shown after electrophoresis and autoradiography. Lane 1, uninfected HeLa NE (54 μg of protein); lane 2, CHO NE (52 μg of protein); lane 3, Hela Ad2-infected cytosol (40 μg of protein); lane 4, HeLa infected cytosol + HeLa NE; lane 5, HeLa infected cytosol + CHO NE; lane 6, CHO Ad2-infected cytosol (68 μg of protein); lane 7, CHO NE and CHO infected cytosol. The letters designate the location of the XbaI fragments on the gel. Fragments D and E did not separate under the conditions of electrophoresis in these experiments. The C and E double-strand fragments represent the right and left termini of Ad2 DNA, respectively; C' and E' are the single strands that have been labeled in vitro during the first round of replication and displaced during the second round (Horwitz and Ariga, in press).*

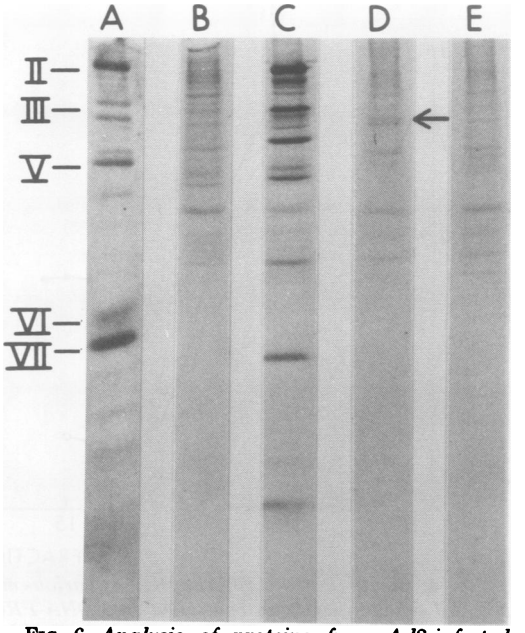

FIG. 6. *Analysis of proteins from Ad2-infected CHO and HeLa cells. A total of 10^6 cells from either HeLa or CHO cell suspension cultures were pulse-labeled with 25 μCi of [^{35}S]methionine for 1 h at various times after infection. The same number of uninfected HeLa and CHO cells were also radioactively labeled with [^{35}S]methionine to serve as controls. After the labeling period, the cells were centrifuged, suspended in 1 ml of electrophoresis buffer, and sonicated (see the text). Portions of each preparation of cells (containing approximately equal numbers of counts per minute) were boiled in the presence of 2% sodium dodecyl sulfate and 1% mercaptoethanol in buffer. The samples were electrophoresed at 100 V for 2.5 h on 12.5% vertical slab gels containing 0.1% sodium dodecyl sulfate until the phenol red dye had reached the bottom of the polyacrylamide slab. The gels were stained with Coomassie brillant blue dye, destained, and dried for autoradiography in a Bio-Rad Gel Dryer. Lane A, Stained gel of purified virion run as marker for the autoradiograms (lanes B to E). Lane B, Uninfected HeLa cells; lane C, Ad2-infected HeLa cells (24 h p.i.); lane D, Ad2-infected CHO cells (36 h p.i.); lane E, uninfected CHO cells. The roman numerals refer to the Ad polypeptides as designated by Maizel (19). The molecular weight of each polypeptide (16) is: II, 120,000; III, 85,000; V, 48,500; VI, 24,000; VII, 18,500. The arrow points to a polypeptide of 75,000 daltons.*

PRO by addition of the DNA polymerase α enzyme is not possible. Until our biochemical reconstructed systems containing various polymerases can be correlated with appropriate cell mutants in these functions, we must cautiously interpret the physiological significance of our DNA polymerase β findings in vitro and continue our search for cellular DNA synthesis mutants.

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