Adenovirus DNA Replication

I. Requirement for Protein Synthesis and Isolation of Nuclear Membrane Fractions Containing Newly Synthesized Viral DNA and Proteins

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Received for publication 10 April 1974

Nuclear membrane fractions were prepared by two procedures from KB cells pulse labeled with [³H]thymidine for 5 min late after infection with adenovirus 2: (i) the M-band technique, which yields a sharp peak containing most of the newly synthesized viral DNA, and (ii) the discontinuous sucrose gradient method, which yields three membrane fractions, one which bands at the interface between sucrose layers at density 1.18 and 1.20 g/ml and contains most of the newly synthesized viral DNA. Studies using cycloheximide to inhibit protein synthesis showed that proteins whose synthesis begins early after infection and occurs in the absence of viral DNA replication are required for viral DNA synthesis late after infection. To study the nature of these proteins, nuclear membrane fractions were isolated from cells labeled with [³H]leucine from 6 to 24 h postinfection in the presence of arabinosyl cytosine to block viral DNA replication, and were analyzed by electrophoresis in sodium dodecyl sulfate polyacrylamide gels. Two proteins of molecular weights 75,000 and 45,000 were the major labeled polypeptides in the nuclear membrane fractions prepared from infected cells both by the M-band and the discontinuous sucrose gradient methods. These two proteins were not found in nuclear membrane fractions from uninfected cells. It is suggested that the 75,000 and 45,000 proteins may be early viral gene products that may play a role in the viral DNA replication.

Investigations of the mechanism of DNA replication and its regulation in mammalian cells are hampered by the complexity of the animal cell and its DNA genome. Based on the general belief that similar mechanisms are involved in the replication of viral and cell DNA, several investigators have used the less complex animal virus DNA genomes as models to study DNA replication. The human adenoviruses are particularly useful for such studies because (i) adenovirus DNA molecules have been well characterized as linear, uninterrupted, non-circularly permuted duplexes of molecular weight 20×10^6 to 25×10^6 (2, 8, 15, 22), and (ii) cell DNA synthesis is completely blocked late after productive infection of KB cells with adenovirus 2, 7, 12, 18, 31 (16), or 5 (5), thus permitting the analysis of viral DNA replication uncomplicated by host cell DNA synthesis.

The availability of subcellular systems capa-

ble of synthesizing adenovirus DNA in vitro would facilitate the analysis of viral DNA replication. Isolated nuclei from adenovirus-infected cells have been shown to synthesize adenovirus DNA in vitro (24), but nuclei are relatively complex organelles. Nuclear membrane fractions derived from adenovirus 2-infected HeLa cells (17) and from adenovirus 12-infected human embryo kidney cells (27) have been reported to be associated with newly synthesized DNA. To evaluate the role of nuclear subfractions in adenovirus DNA replication and their possible use for the analysis of viral DNA replication, we studied the properties and protein components of nuclear membrane fractions isolated from adenovirus-infected KB cells. We show here that nuclear membrane fractions isolated by the M-band and by the discontinuous sucrose gradient procedures contain newly synthesized viral DNA and two major polypeptides of molecular weights 75,000 and 45,000. These proteins are synthesized in the absence of viral DNA synthesis and could be

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either early viral gene products or derepressed cellular proteins that are involved in DNA replication.

After completion of these studies, we learned from A. Levine in September 1973 of two proteins of similar size isolated on DNA cellulose columns from monkey kidney cells abortively infected with adenovirus 5 (23). The 75,000 and 45,000 polypeptides that we isolated from nuclear membrane fractions also bind to DNA-cellulose columns. (G. Shanmugam, S. Bhaduri, M. Arens, and M. Green, in manuscript in preparation), suggesting that they are related to the proteins in adenovirus 5-infected monkey cells (23). Of further interest, a membrane fraction containing these two proteins can synthesize adenovirus DNA sequences in vitro (T. Yamashita, M. Arens, and M. Green, manuscript in preparation).

MATERIALS AND METHODS

Cell culture and virus infection. A clonal KB cell line was grown in suspension in Eagle minimal essential medium (MEM) containing 5% horse serum. For infection, cells were centrifuged and resuspended at 5×10^6 cells/ml in medium without serum, and adenovirus 2 (strain 38-2, plaque 4, free of adenovirus-associated viruses) was added at a multiplicity of 100 PFU/cell (4). After 1 h of absorption at 37 C, the cell suspension was diluted with complete medium to 3×10^6 cells/ml and further incubated in suspension at 37 C.

Labeling of nascent DNA in infected cells. Monolayer cultures of KB cells growing in MEM with 10% calf serum were infected at 36 C. Cells were labeled with [3 H]thymidine (New England Nuclear, 6.7 Ci/mmol, 10 μ Ci/ml) at 18 h postinfection, a time when viral DNA synthesis is maximal (6). After a 5-min pulse, some cultures were harvested immediately while parallel cultures were washed and further incubated in medium containing unlabeled 0.2 mM thymidine. Cells were harvested after various times of chase with unlabeled thymidine as indicated in the text.

Preparation of nuclear membrane fractions. Adenovirus 2-infected and mock-infected KB cells, harvested in various experiments, were washed with phosphate-buffered saline lacking Mg^{2+} and Ca^{2+} (3), suspended in sterile reticulocyte standard buffer (RSB: 0.010 M NaCl, 0.01 M Tris-hydrochloride [pH 7.4], 0.0015 M $MgCl_2$) at 10^6 cells/ml, and placed in an ice bath. After 15 min, cells were disrupted with 15 to 20 strokes of a tight-fitting Dounce homogenizer (Kontes Glass), and nuclei were centrifuged through 5 ml of a 25% sucrose cushion in RSB in 50-ml tubes, at $2000 \times g$ for 10 to 15 min in an International PR-2 centrifuge at 4 C. The nuclear pellet was washed with cold RSB, centrifuged, and resuspended at 10^6 nuclei/ml in Tris-Mg-K buffer (0.10 M Tris-hydrochloride [pH 7.5], 0.01 M $MgCl_2$, 0.1 M KCl) or at 10^6 to 10^7 nuclei/ml in TKM buffer (0.05 M Tris-hydrochloride

[pH 7.5], 0.005 M $MgCl_2$, 0.025 M KCl, 0.003 M dithiothreitol) for the isolation of nuclear membrane fractions by a modification of the M-band technique (21, 27) or by the discontinuous sucrose density gradient procedure (13, 27), respectively. To isolate the M-band, the nuclear suspension was lysed by gently mixing with an equal volume of 0.2% sodium *N*-lauroyl sarcosinate, layered over 4 ml of 15% sucrose above 6 ml of 40% sucrose in Tris-MgK buffer, and centrifuged in a SW41 rotor for 20 min at 20,000 rpm. To isolate nuclear membrane fractions by the discontinuous sucrose gradient procedure, the nuclear suspension in TKM buffer was sonically disrupted at full power in a Raytheon DF-101 sonic oscillator at 0 C (usually 2 min) until over 98% of the cells were broken, as monitored by phase microscopy. Sodium citrate was added to 10%, and the suspension was layered over a discontinuous gradient containing from top to bottom 1.16 to 1.18, 1.18 to 1.20, and 1.20 to 1.22 g of sucrose per ml in TKM buffer containing 10% sodium citrate. The tubes were centrifuged at 4 C in a Spinco SW27 rotor for 60 min at 25,000 rpm.

DNA-DNA hybridization. Adenovirus DNA or KB cell DNA in $6 \times$ SSC (SSC: 0.15 M NaCl-0.015 M sodium citrate) containing 0.1% sodium dodecyl sulfate (SDS) was annealed at 60 C for 24 h and processed as described (7).

Zonal centrifugation in alkaline sucrose gradients. Labeled DNA in 2 ml of 0.5 N NaOH, 0.1 M EDTA was layered over a 28-ml linear 5 to 20% sucrose density gradient in 0.3 N NaOH, 0.5 M NaCl, 0.01 M EDTA, and centrifuged at 15 C in a Spinco SW25.1 rotor at 22,000 rpm for 18 h. One-milliliter fractions were collected from the bottom of the tube, bovine serum albumin (50 μ g) and 0.1 volume of 50% trichloroacetic acid were added, and the acid-insoluble precipitate was collected on membrane filters and counted in 10 ml of toluene-based scintillation fluid.

Polyacrylamide gel electrophoresis of polypeptide components of nuclear membrane fractions. At 2 h after infection of KB suspension cultures with adenovirus 2, 25 μ g of 1- β -D-arabinofuranosylcytosine (AraC) per ml was added. The culture fluid was replaced at 6 h with MEM lacking leucine and containing 25 μ g of AraC and 2 μ Ci of [3 H]leucine (20 Ci/mmol, New England Nuclear) per ml, and the cells were incubated until 24 h after infection. Nuclear membrane fractions were prepared by the M-band or by the discontinuous sucrose density gradient methods and solubilized by heating at 100 C for 1 min in 0.01 M phosphate buffer (pH 7.2) containing 2% SDS and 1% 2-mercaptoethanol. The solubilized polypeptides were resolved by electrophoresis on gels (0.45 by 7 cm) containing 7.5% recrystallized polyacrylamide, 0.2% recrystallized bisacrylamide, 0.1% *N,N,N',N'*-tetramethylene-diamine, 0.14% ammonium persulfate, and 0.2% SDS in 0.05 M phosphate buffer (pH 7.2). Electrophoresis was performed in 0.01 M phosphate buffer (pH 7.2) containing 0.1% SDS at 5 mA per gel for 6 h. Gels were sliced into 1.0-mm fractions and incubated with 0.3 ml of 30% H_2O_2 in scintillation vials at 60 C overnight. Ten milliliters of aquasol (New England Nuclear) was added and radioactivity was measured in a scintillation spectrometer.

RESULTS

Association of newly replicated DNA in adenovirus-infected cells with nuclear membrane fractions. To determine whether newly synthesized viral DNA is associated with the nuclear membrane, adenovirus 2-infected KB cells were pulse labeled for 5 min with [^3H]thymidine at 18 h after infection and nuclear membrane fractions were prepared by the M-band and discontinuous sucrose gradient methods. The M-band procedure was described initially by Tremblay et al. (21) for the preparation of membrane DNA-RNA complexes from *B. subtilis*, and was subsequently used to isolate nuclear membrane fractions containing newly synthesized DNA from HeLa cells (9) and sea urchins (12). In this procedure, nuclei were lysed with sodium dodecyl sarcosinate in the presence of Mg^{2+} , and a nuclear membrane fraction containing newly synthesized DNA was co-precipitated with magnesium sarcosinate crystals and isolated by zonal centrifugation. About 90% of the pulse-labeled DNA in adenovirus-infected cells was found as a sharp peak at the position of the visible M-band at the interface between the 15 and 40% sucrose layers (Fig. 1).

In the discontinuous sucrose gradient procedure of Kashing and Kasper (13), nuclei were sonically disrupted and treated with 10% sodium citrate, and nuclear membrane fractions were separated by sedimentation in a discontinuous sucrose gradient. Three fractions containing newly synthesized DNA were isolated at the interface between densities 1.16 to 1.18, 1.18 to 1.20, and 1.20 to 1.22 g/ml (Fig. 2). Similar patterns were reported for rat liver and HeLa cells by Mizuno et al. (14). The highest content of newly synthesized labeled [^3H]DNA was found at the 1.18 to 1.20 g/ml interface, the position reported for the inner nuclear membrane (14); much smaller quantities of labeled DNA were found at the 1.16 to 1.18 and 1.20 to 1.22 g/ml interface regions of the gradient.

Origin of nascent DNA associated with M-band. By DNA-DNA hybridization, newly synthesized DNA associated with the M-band was shown to consist predominantly of viral base sequences. As shown in Table 1, 54% of the DNA labeled during a 5-min pulse with [^3H]thymidine at 18 h after infection hybridized with adenovirus 2 DNA. A similar efficiency of hybridization was found with authentic adenovirus 2 [^3H]DNA. Less than 1% of the labeled DNA associated with M-band annealed with KB cell DNA (Table 1).

Release of viral DNA from the M-band. The fate of newly synthesized viral DNA as-

FIG. 1. Association of newly synthesized DNA with the nuclear M-band. Nuclei prepared from 18-h infected KB cells pulse labeled with [^3H]thymidine for 5 min were treated with sodium dodecyl sarcosinate in the presence of MgCl_2 . After centrifugation at 20,000 rpm for 20 min in a Spinco SW41 rotor onto a two-layer discontinuous gradient consisting of 15 and 40% sucrose, 0.5-ml fractions were collected from the top and acid-precipitable radioactivity was determined.

sociated with the M-band was followed during a chase with unlabeled thymidine. The amount of radioactive DNA associated with the M-band decreased with time and reached a plateau of 30% of the total radioactivity in the gradient after a 30-min chase with cold thymidine (Fig. 3). After a 60-min chase, [^3H]DNA present at the top of the gradient above the M-band cosedimented with the adenovirus DNA marker at 34S (8) in an alkaline sucrose gradient (Fig. 4). These results suggest that adenovirus DNA replicates in association with the M-band fraction and is released as mature viral DNA.

Effect of inhibiting protein synthesis on DNA replication in uninfected and adenovirus 2-infected KB cells. Polasa and Green in 1965 (18) described evidence for the synthesis of proteins at about 4 h after infection of KB cells by adenovirus 2 that were required for replica-

FIG. 2. Association of newly synthesized DNA with nuclear membrane fractions isolated by the discontinuous sucrose gradient procedure. Nuclei prepared from KB cells pulse labeled with [^3H]thymidine for 5 min at 18 h postinfection were sonically disrupted and fractionated by the discontinuous sucrose gradient procedure. After centrifugation for 60 min at 25,000 rpm in a Spinco SW27 rotor, 1.0-ml fractions were collected and acid-precipitable radioactivity was determined.

TABLE 1. Hybridization of labeled DNA associated with the nuclear membrane fraction of adenovirus 2-infected cells with adenovirus DNA and KB cell DNA

Input DNA	Immobilized DNA (10 μg) from	Bound DNA	
		Counts/min	%
Nuclear membrane associated [^3H]DNA (3,320 counts/min)	Adenovirus 2	1,775	53.5
	KB	20	0.7
Adenovirus 2 [^3H]DNA (13,640 counts/min)	Adenovirus 2	7,665	56.2
	KB	0	0

tion of viral DNA, beginning at about 6 to 7 h after infection. Inhibition of protein synthesis from 2 to 13 h postinfection inhibited viral DNA replication by 97%, indicating an absolute requirement for viral DNA replication of early protein synthesis (18). Inhibition of protein synthesis by *p*-fluorophenylalanine or puromycin after viral DNA synthesis had begun was

much less effective. Based on studies with a temperature-sensitive mutant of adenovirus 31, Suzuki and Shimojo (19) suggested that proteins other than enzymes may be required for adenovirus DNA replication. To analyze further the requirement for protein synthesis during adenovirus DNA replication, cycloheximide (25 $\mu\text{g}/\text{ml}$) was added to KB cells at 20 h after infection, and the subsequent incorporation of [^3H]thymidine into DNA was followed. Cycloheximide effectively blocked protein synthesis, incorporation of [^3H]leucine being inhibited by 85% (Fig. 5). However, DNA synthesis in adenovirus-infected cells was not blocked but continued at about the same rate as that of untreated cultures for the next 5 h. In contrast, DNA synthesis was suppressed 80 to 90% in uninfected cells at 1 h after treatment with cycloheximide (data not shown). Horwitz et al. (10) recently reported similar findings for adenovirus 2-infected HeLa cells.

The above results suggest that although con-

FIG. 3. Release of nascent viral DNA from the M-band. KB cells were pulse labeled for 5 min at 18 h postinfection with [^3H]thymidine, and radioactivity was then chased for various times in the presence of unlabeled 0.2 mM thymidine. M-band preparations were isolated from nuclei at different times and acid-precipitable radioactivity was determined. Results are expressed as percentage of the total radioactivity recovered from the gradient.

FIG. 4. Rate-zonal centrifugation in alkaline sucrose gradients of pulse-labeled DNA released from the M-band. The M-band was isolated from the nuclei of KB cells pulse labeled with [^3H]thymidine for 5 min at 18 h postinfection and chased with unlabeled 0.2 mM thymidine for 60 min. [^3H]DNA was isolated in the gradient above the M-band by phenol extraction and ethanol precipitation and was sedimented in alkaline sucrose gradients together with marker adenovirus 2 [^{14}C]DNA.

tinued protein synthesis is necessary for the replication of cellular DNA, it is not essential for the synthesis of adenovirus DNA. If protein synthesis is needed for adenovirus DNA replication, this must occur before 20 h after infection, and the proteins must be more stable than those required for cellular DNA synthesis. Evidence that early proteins are required late after infection for adenovirus DNA replication was obtained by treating KB cells with cycloheximide at 2 h and testing their ability to synthesize DNA at 20 h postinfection. Incorporation of [^3H]thymidine into DNA was inhibited by 90% (data not shown). In similar studies, Horwitz et al. (10) reported that inhibition of protein synthesis in HeLa cells up to 9 h after infection with adenovirus 2 blocked viral DNA synthesis.

To further distinguish the relationship between early and late viral gene functions and the proteins required for viral DNA replication,

the experiments described in Fig. 6 were performed. AraC was added to KB cells at 2-h postinfection to block viral DNA synthesis and thus the synthesis of late viral proteins (25). At 24-h postinfection, AraC was removed, unlabeled deoxycytidine was added to reverse the inhibitory effect of AraC, cycloheximide was added to block protein synthesis, and [^3H]thymidine was used to measure DNA synthesis. DNA synthesis in uninfected cells was inhibited by cycloheximide and blocked completely by 3 to 4 h after treatment. In contrast, DNA synthesis in adenovirus-infected cells continued at the same rate in cycloheximide-

FIG. 5. Effect of inhibition of protein synthesis in late adenovirus-infected cells on DNA synthesis. Cycloheximide (25 $\mu\text{g}/\text{ml}$) was added to adenovirus 2-infected KB cells at 20 h postinfection and the rate of DNA and protein synthesis was analyzed by continuous labeling with [^3H]thymidine (2 $\mu\text{Ci}/\text{ml}$, 6.7 Ci/mmol) and [^3H]leucine (2 $\mu\text{Ci}/\text{ml}$, 5 Ci/mmol), respectively. Cells were harvested every 60 min and acid-precipitable radioactivity was determined. Symbols: (Δ) DNA synthesis in untreated cells; ($\blacktriangle$) DNA synthesis in cells treated with cycloheximide; ($\circ$) protein synthesis in untreated cells; ($\bullet$) protein synthesis in cells treated with cycloheximide.

FIG. 6. Effect of inhibition of protein synthesis on DNA synthesis in uninfected and adenovirus 2-infected KB cells after treatment with AraC. AraC was added to adenovirus 2-infected and uninfected KB cell cultures at 2 h postinfection. At 24 h postinfection, AraC was removed and cycloheximide (25 μ g/ml), deoxycytidine (50 μ g/ml), and [3 H]thymidine (2 μ Ci/ml) were added. Cells were harvested every 60 min and acid-precipitable radioactivity was measured. Symbols: (○) Uninfected cells without cycloheximide; (●) uninfected cells with cycloheximide; (Δ) adenovirus 2-infected cells without cycloheximide; (▲) adenovirus 2-infected cells with cycloheximide.

treated as in untreated cells for at least 6 h. We conclude that protein(s) required for adenovirus DNA replication are synthesized early during infection and could represent early viral gene function(s).

Polyacrylamide gel electrophoresis of proteins in the nuclear membrane complex isolated from adenovirus-infected cells. Since nascent adenovirus DNA appears to be associated with the M-band (Fig. 1) and the inner nuclear membrane (Fig. 2) fractions, and newly synthesized proteins are necessary for viral

DNA synthesis, we analyzed the nuclear membrane fractions for the presence of newly synthesized proteins. Cells were treated with AraC at 2 h postinfection to suppress the synthesis of late viral proteins (Shanmugam et al. manuscript in preparation) and were then labeled with [3 H]leucine from 6 to 20 h postinfection. Nuclear membrane fractions were isolated by both the M-band and discontinuous sucrose gradient methods, dissociated by treatment with SDS and β -mercaptoethanol together with [14 C]leucine labeled adenovirus to provide polypeptide molecular weight markers, and electrophoresed on SDS gels. The M-band from infected cells contained two major labeled polypeptides of molecular weights 75,000 and 45,000 (Fig. 7). Smaller peaks of polypeptides approximately 27,000 and 15,000 and heterogeneous higher- and lower-molecular-weight polypeptides were

FIG. 7. Co-electrophoresis of [3 H]leucine-labeled M-band polypeptides from adenovirus 2-infected KB cells with [14 C]leucine-labeled adenovirus 2 polypeptides. An M-band preparation from adenovirus 2-infected KB cells treated with AraC at 2 h and labeled with [3 H]leucine from 6 to 24 h postinfection was disrupted together with [14 C]leucine-labeled adenovirus 2 using SDS and β -mercaptoethanol. Electrophoresis was performed on SDS gels.

also detected. To determine whether similar proteins were present in uninfected cells, [^3H]leucine-labeled M-band polypeptides from infected cells were co-electrophoresed with [^{14}C]leucine-labeled M-band polypeptides from uninfected cells. The polypeptides of uninfected cells were heterogeneous, ranging from 10,000 to over 120,000 daltons (Fig. 8). The distinct 75,000 and 45,000 polypeptide peaks observed in the infected cell M-band were not present in appreciable amounts in uninfected cell M-band preparations. The same two major polypeptides were present also in the nuclear membrane fraction (density interface 1.18 to 1.20 g/ml) isolated from adenovirus 2-infected cell nuclei by the discontinuous sucrose gradient method (Fig. 9). The similar polypeptide pattern indicates that both procedures yield similar subnuclear preparations. The possibility that the smaller polypeptides are degradation products of the 75,000 molecular weight protein is not excluded.

DISCUSSION

The replication of adenovirus 2 DNA in KB cells late after infection can occur under conditions of inhibition of late viral protein synthesis, as shown here and by Horwitz et al. (10).

FIG. 8. Co-electrophoresis on SDS gels of [^3H]leucine-labeled M-band polypeptides from adenovirus 2-infected KB cells with [^{14}C]leucine-labeled M-band polypeptides from uninfected KB cells.

FIG. 9. Coelectrophoresis on SDS gels of [^3H]leucine-labeled polypeptides of the nuclear membrane fraction at the density between 1.18 and 1.20 g/ml isolated by the discontinuous sucrose gradient procedure together with [^{14}C]leucine-labeled adenovirus 2 polypeptides.

Protein(s) that is synthesized early during infection in the absence of viral DNA synthesis and presumably representing early viral gene functions is essential to and sufficient for the subsequent replication of adenovirus DNA. To search for such early proteins that function in adenovirus DNA replication, we isolated nuclear membrane fractions that contained newly synthesized viral DNA from adenovirus-infected cells by two different procedures. Analysis by SDS-polyacrylamide gel electrophoresis revealed two major polypeptides of molecular weights, 75,000 and 45,000, not present in appreciable amounts in nuclear membrane fractions from uninfected KB cells. These two polypeptides were synthesized under conditions of blocked viral DNA and virion protein synthesis, and are distinct from virion polypeptides of similar molecular weights, as shown by co-electrophoresis on high-resolution polyacrylamide gels (Shanmugan, Bhaduri, Arens, and Green, unpublished data).

The functions of the 75,000 and 45,000 polypeptides are unknown. They are similar in size to the DNA-binding proteins recently isolated from adenovirus 5-infected monkey kidney cells by van der Vliet and Levine (23), and also bind to DNA-cellulose columns (Shanmugan et al.

unpublished data). Of further interest, the size of the 75,000 molecular weight polypeptide corresponds in coding capacity to the DNA strand-specific mRNA molecules of molecular weight 0.7×10^6 recently isolated from the polyribosomes of KB cells early after infection, suggesting that it may be viral coded (W. Büttner, Z. Veres-Molnar, and M. Green, Proc. Nat. Acad. Sci. U.S.A., in press). Of additional interest, some of the polypeptides in the nuclear membrane fractions are similar in size to two of the T-antigens detected in adenovirus 12-transformed cells and in adenovirus 12-infected KB cells (20).

The role of the nuclear membrane in the replication of cellular DNA is controversial. Several studies have found an association of nuclear membrane fractions with newly synthesized DNA by biochemical methods (9, 12, 14), whereas others have failed to find evidence for an association by cytological procedures (1, 11, 26). In the present study, two nuclear membrane fractions were isolated from adenovirus 2-infected cells by established procedures and were shown to contain newly synthesized DNA. The nuclear membrane preparation isolated by the discontinuous sucrose gradient procedure in additional studies was shown to synthesize adenovirus DNA sequences in vitro and to possess both DNA polymerase and DNA endonuclease activity (Yamashita, Arens, and Green, manuscript in preparation). Although these results do not establish that the nuclear subfractions isolated from adenovirus infected cells function to replicate DNA within the cell, they do provide biochemical preparations that are useful for the further analysis of the enzymes and other components involved in the replication of adenovirus DNA.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI-01725 from the National Institute of Allergy and Infectious Diseases and from Contract NO1 CP 70692 from the Virus Cancer Program of the National Cancer Institute.

M.G. is a Research Career Awardee of N.I.H. (5K6-AI-4739).

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