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Lynne A. Lucher

Illinois State University

Benjawan Khuntirat

Illinois State University

Jiansheng Zhao

Illinois State University

Peter C. Angeletti

Illinois State University, pangeletti2@unl.edu

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Altered Expression of Adenovirus 12 DNA-Binding Protein but Not DNA Polymerase during Abortive Infection of Hamster Cells

Lynne A. Lucher, Benjawan Khuntirat,* Jiansheng Zhao,
and Peter C. Angeletti

Department of Biological Sciences, Illinois State University, Normal, Illinois, USA

Corresponding author – Lynne A. Lucher, Department of Biological Sciences, Illinois State University, Normal, Illinois, 61761 USA

* *Present address* – Department of Microbiology and Immunology, Indiana University Medical Center, Indianapolis, Indiana, USA

Abstract

Replication of human adenovirus type 12 DNA is blocked in abortively infected baby hamster kidney cells. The activity and accumulation of adenovirus 12 DNA polymerase is equivalent in infected hamster and human cell extracts. However, the accumulation of adenovirus type 12 DNA-binding protein is approximately 120-fold lower in extracts from infected hamster cells when compared to infected permissive human cells. This difference in accumulation is not because of replication of viral DNA during productive infection, since this difference is observed in the presence of hydroxyurea. The DNA-binding protein from infected hamster cells retains the ability to bind denatured DNA-cellulose. An adenovirus 5 early region 1 transformed hamster cell line competent to complement the adenovirus 12 DNA replication defect also stimulates accumulation of the DNA-binding protein even when the cells are treated with hydroxyurea. Thus, the reduced expression of the viral DNA-binding protein may play a role in the mechanism of abortive infection of hamster cells by adenovirus 12.

Introduction

Successful virus infection of a host cell relies on complex interactions between viral and cellular proteins and nucleic acids. The nature of these interactions can define whether an infection will be productive or abortive for a particular cell type or species. For example, human cells support a productive infectious cycle of human adenovirus type 12 (Ad12), while hamster cells fail to support complete infection.

Ad12 infection of hamster cells is aborted because viral DNA replication does not occur (Doerfler, 1969). Ad DNA replication requires the viral proteins DNA-binding protein (DBP), DNA polymerase (DNApol), and terminal protein precursor (pTP), plus the cellular nuclear factors I (NFI/CTF), II, and III (NFIII/OTF-1) (reviewed in Challberg and Kelly, 1989). Hamster cell nuclear factors are able to participate in the initiation step of Ad 12 DNA replication *in vitro*, although evidence suggests that they may be less efficient in supporting the reaction than human nuclear factors (Chowrira and Lucher, 1990).

One hypothesis for explaining abortive infection is that expression of at least one of the Ad 12 replication proteins is too low to support viral DNA replication. Although early genes are transcribed (Ortin et al., 1976), synthesis of the E1A and E1B transforming proteins is reduced during abortive infection (Lucher, 1990). On the other hand, the activities of DNApol and pTP for the initiation of DNA replication *in vitro* are equivalent in productive and abortive infection (Chowrira and Lucher, 1990). In this paper, we present evidence that expression of Ad12 DBP is reduced dramatically during abortive infection. We also present evidence that suggests the importance of this reduced expression; complementation by Ad5 E1 genes of Ad 12 DNA replication in hamster cells also stimulates DBP expression.

Materials and Methods

Cells and virus

Human KB cells, baby hamster kidney (BHK-21 C13) cells, and adenovirus type 12 (Ad12, strain Huie) were from the American Type Culture Collection. The Ad5 early region 1 transformed hamster cell line 983.2 (Rowe et al., 1984) was a gift from Frank L. Graham, McMaster University. KB cell monolayers were maintained in MEM with 10% calf serum, while BHK and 983.2 cell monolayers were maintained in Dulbecco's MEM (DMEM) with 10% fetal bovine serum. Particle concentration of stock Ad 12 was calculated based on optical density (Lucher, 1990).

Preparation of cell-free extracts

Monolayer cultures of BHK and 983.2 cells were typically infected with three times the Ad12 m.o.i. used for KB cells; this compensates for the reduced efficiency of hamster cell infection by Ad 12 (Lucher, 1990). In some cases, the cells were infected in the presence of 10 mM hydroxyurea. For immunoprecipitation analysis, the cells were labeled either with 500 μ Ci/ml Trans 35 S-label (> 1100 Ci/mmol; ICN Radiochemicals) for 2 to 3 hr following starvation in methionine-free medium for 1 hr or 5 mCi/ml 32 Pi (ICN Radiochemicals) for

12 hr following starvation in phosphate-free medium for 3 hr. Mock infections were performed as negative controls. Cells were harvested into sonication buffer (20 mM Tris (pH 7.4), 0.5 M urea, 50 mM NaCl, 5 mM Na₄EDTA, 1 mM mercaptoethanol, 10% glycerol, 1% sodium deoxycholate, 0.5% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, and 100 µg/ml aprotinin) and the cells broken by sonication for 2 min in a Branson sonifier using an ice-water-cooled cup horn. Alternatively, the cells were fractionated into cytoplasmic and nuclear extracts using Nonidet P-40 as described previously (Lucher et al., 1986). The nuclei were either suspended in sonication buffer and lysed by sonication (Nonidet P-40/sonication method) or suspended in 50 mM HEPES (pH 7.5), 10% glycerol, and incubated with 0.3 M NaCl to prepare nuclear extracts (Nonidet P-40/NaCl method) (Chowrira and Lucher, 1990). Radiolabel incorporation into proteins was determined by trichloroacetic acid (TCA) precipitation and liquid scintillation counting.

For immunoblotting, unlabeled infected and mock-infected cell extracts were prepared by one of the above methods. Protein concentrations of extracts were determined after TCA-deoxycholate coprecipitation (Khuntirat and Lucher, 1990).

Antipeptide antisera

Peptide 4 (HSQRERTPDRS) was synthesized by BioSearch, Inc., and corresponds to residues 6 through 16 of the predicted amino acid sequence of Ad12 DBP (Kruijer et al., 1983). A C-terminal cysteine residue was included in peptide 4 to facilitate coupling to carrier protein. Peptide 4 was coupled to keyhole limpet hemocyanin (KLH) via the C-terminal cysteine residue, and antisera were raised against the peptide-KLH conjugate, as described previously (Green et al., 1983).

Other antisera used have been described elsewhere: antipeptide 6 antiserum, targeted to the Ad 12 DNApol C-terminal sequence PNPREEVC (Chowrira et al., 1991), and antipeptide 8 antiserum, targeted to the Ad12 E1B 163R C-terminal sequence QEEKEEERN-PAVVEK (Lucher, 1990).

Immunoprecipitation and immunoblot analysis of extracts

Aliquots of radiolabeled extracts containing 5×10^6 to 2×10^7 TCA-precipitable cpm were analyzed by immunoprecipitation with 10 µl antipeptide antiserum (Green et al., 1979); in some cases, extract aliquots were first precleared by immunoprecipitation with 10 µl pre-immune rabbit serum. For competition analysis, 10 µg homologous peptide per ml was included in the immunoprecipitation reaction containing antipeptide antiserum. Immunoprecipitated proteins were fractionated by discontinuous SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 14% gels and the proteins visualized by fluorography of sodium salicylate-treated gels using Kodak X-Omat film (Lucher et al., 1986). Prestained molecular weight markers (SDS-7B mixture; Sigma Chemical Co.) were routinely included in gels to monitor electrophoresis. However, the molecular weight of DBP was determined using nonprestained standard proteins, due to possibly anomalous mobilities of the prestained markers (Sigma Technical Bulletin MWS-877PSB).

For immunoblot analysis, aliquots of unlabeled extracts were fractionated by SDS-PAGE, or by nondenaturing PAGE, and the proteins were electrophoretically transferred to nitrocellulose membrane (Lucher and Lego, 1989). For nondenaturing electrophoresis,

the SDS-PAGE system was modified to exclude SDS from all buffers, and the protein samples were mixed with a solution containing 10% glycerol and 0.02% bromophenol blue. Protein blots were blocked in Tris-buffered saline (TBS; 10 mM Tris (pH 7.4), 0.9% NaCl) plus 5% nonfat dry milk. Blots were incubated in sequence with anti-peptide antiserum in TBS plus 5% BSA, TBS, and peroxidase-conjugated goat anti-rabbit IgG antibody (Sigma Chemical Co.) in TBS plus 0.05% Tween 20. Immune complexes were visualized with a solution containing 3 mg luminol (3-aminophthalhydrazide), 3 mg 4-iodophenol, and 60 μ l H₂O₂ in 50 ml 0.1 M Tris, pH 8.0 (Vachereau, 1989) and chemiluminescence recorded by exposure to X-ray film. Alternatively, blots were incubated with a freshly prepared solution containing 30 mg 4-chloro-1-naphthol in 10 ml methanol, 50 ml TBS, and 50 μ l H₂O₂ to visualize antibody:antigen complexes. For peptide competition, antiserum was incubated with homologous peptide (1 mg/ml undiluted serum) for at least 2 hr at 4° prior to incubation with the blot.

Densitometric analysis of autoradiographs and immunoblots was performed with a Bioimage Visage 110 image analyzer (Kodak). For quantitation from X-ray films, integrated intensities of individual protein bands were determined as OD $\times$ mm² using conditions of linear film response to radioactivity or chemiluminescence.

DBP binding to DNA-cellulose

Binding of Ad12 DBP to denatured DNA-cellulose was determined by analytical batch chromatography in microcentrifuge tubes or by column chromatography in 1-ml syringe barrels (Khuntirat and Lucher, 1990).

Ad12 DNApol activity

DNApol activity was assayed in cytoplasmic extracts, and in nuclear extracts prepared with 0.3 M NaCl. Activity was determined at 37° by incubating cell extract (20 μ g protein) and DNA template in a 50- μ l reaction mixture containing 50 mM Tris (pH 7.5), 2 mM ATP, 5 mM MgCl₂, 0.5 mM DTT, and either 33 pmol (2 μ Ci) [³H]TTP or 58 pmol [γ -³²P]TTP (10 μ Ci). To maximize the specificity of the reaction for Ad12 DNApol in contrast to cellular DNApol alpha, poly(dA) plus oligo(dT) (dA:dT) was used as template instead of activated calf-thymus DNA (Field et al., 1984). Incorporation of label into dA:dT was determined by TCA precipitation followed by scintillation counting. The Ad12 DNApol activity was calculated by subtracting the mock-infected sample dpm from the infected sample dpm.

DNA isolation from infected cells

Total DNA was isolated from whole cells in a manner that reduced shearing (Ausubel et al., 1987). To avoid detecting integrated Ad5 E1 DNA when samples were from 983.2 cells, Ad12 DNA was detected by Southern hybridization of either undigested or restriction enzyme-digested DNA (Maniatis et al., 1982) using plasmid gE4-2, which contains the Ad12 E4 region (Shiroki et al., 1984).

Results

Preparation and characterization of anti-peptide antiserum to DBP

The antiserum to peptide 4 (Ab4) immunoprecipitated a 58K phosphoprotein from Ad12-infected extracts (Fig. 1A). Preincubation of Ab4 with homologous peptide (Fig. 1A), but not heterologous peptide (not shown), prevented immunoprecipitation of the 58K protein. Ab4 also reacted specifically with a 58K protein in immunoblots of infected, but not mock-infected, extract (Fig. 1B). The 58K protein bound to single-stranded DNA-cellulose (Fig. 1C) and was close in molecular weight to the 60K DNA-binding protein previously detected in extracts of Ad12-infected cells (Rosenwirth et al., 1975).

Figure 1. Detection of the AD12 DBP with anti-peptide antiserum (Ab4). (A) Detection of DBP by immunoprecipitation. Aliquots of ^{35}S - and ^{32}P -labeled crude extracts of KB cells infected for 33 hr were incubated with prebleed serum (N) or with Ab4 in the absence ($\alpha 4$) or presence ($\alpha 4 + P$) of 10 μg homologous peptide. Hatch marks on the left show the positions of marker proteins (top to bottom) of 84K, 58K, and 48.5K molecular weight. (B) Detection of DBP by immunoblotting. Crude extracts (60 μg aliquots) of KB cells infected for 36 hr (I) or mock infected (M) were analyzed. Blots were incubated with Ab4 in the absence ($\alpha 4$) or the presence ($\alpha 4 + P$) of competing peptide. Immune complexes were visualized with 4-chloronaphthol; the arrowhead indicates the DBP. (C) Binding of DBP to denatured DNA-cellulose. Crude extracts of ^{35}S -labeled KB cells infected for 44 hr were subjected to batch chromatography with denatured DNA-cellulose. Bound proteins were eluted with 1 M NaCl. Aliquots of unbound (U) or eluted (E) proteins containing 5×10^4 TCA-precipitable cpm were fractionated directly by SDS-PAGE (TOTAL), and aliquots containing 6×10^4 TCA-precipitable cpm were immunoprecipitated with Ab4 (IP).

Synthesis of Ad12 DBP during abortive infection

The synthesis of DBP in BHK cells was not detectable by immunoprecipitation of either ^{35}S -labeled or ^{32}P -labeled extracts (not shown). Immunoblot analysis revealed barely detectable accumulation of DBP in whole cell extracts by 48 hr p.i., with sufficient Ad12 m.o.i. (Fig. 2A). Comparison of the amount of detectable DBP in BHK extract with that observed in KB extract from equivalent infections (Lucher, 1990) demonstrated that DBP accumulation was approximately 200-fold greater in KB (Fig. 2B). Because DBP expression increases after DNA replication begins, we tested accumulation in the presence of hydroxyurea to block viral DNA replication in KB. Inclusion of 10 mM hydroxyurea during a 48-hr infection (Challberg and Kelly, 1979) decreased DBP accumulation in KB cells approximately 8-fold (Fig. 2C). Thus in the absence of DNA replication, DBP levels were about 120-fold more in KB than in BHK cells. It is likely that DBP was localized correctly during abortive infection, since DBP was found in nuclear extracts of BHK cells (Fig. 2D).

Figure 2. Accumulation of Ad12 DBP during productive and abortive infection. In all cases, DBP was detected by immunoblotting with Ab4, and immune complexes were visualized using luminol. (A) DBP accumulation with increasing Ad12 m.o.i. KB and BHK cells were infected for 48 hr with increasing m.o.i. (given as number of Ad12 particles/cell), and crude extract from KB (60 μg protein) or BHK (120 μg protein) infections were analyzed. Mock-infected extract (0 m.o.i.) was used as a negative control. (B) Relative quantitation of DBP in BHK extract. The DBP present in extracts from infections

shown in part A was compared; BHK extract from the 4035 particles/cell infection and KB extract from the 1345 particles/cell infection were used. (C) Effect of hydroxyurea on DBP accumulation in infected KB cells. Crude extracts from 48-hr infections of KB cells in the absence (-HU) and the presence (+HU) of hydroxyurea were analyzed for relative levels of DBP. (D) Distribution of DBP in cell fractions. Infected and mock-infected KB and BHK cells were harvested at 48 hr p.i. and fractionated by the Nonidet P-40/sonication method; aliquots of cytoplasmic (C) or nuclear (N) extracts containing 50 μ g protein from KB, or 100 μ g protein from BHK, were analyzed.

Ad12 DBP binding to DNA cellulose

DBP binds to single-stranded viral DNA during replication, a function that is reflected by its ability to also bind to nonspecific denatured DNA (Rosenwirth et al., 1975; Khuntirat and Lucher, 1990). DBP synthesized in BHK cells bound to denatured calf-thymus DNA-cellulose, but eluted at lower NaCl concentrations than DBP synthesized during productive infection (Fig. 3).

Figure 3. DNA-binding activity of Ad12 DBP from infected KB and BHK nuclear extracts. Chromatography on a 0.4-ml column of denatured DNA-cellulose was performed with unlabeled crude extracts from KB (275 μ g protein) or BHK (1.6 mg protein) cells infected for 48 hr. Extract was applied at 0.05 M NaCl, and bound proteins were removed by step elution with 1.5 ml of buffer containing 0.1, 0.25, 0.5, or 1 M NaCl. The entire aliquot of each elution, 1/3 volume of unbound (0.05 M NaCl) KB protein and 1/5 volume of unbound BHK protein, were concentrated by deoxycholate-TCA precipitation and analyzed by immunoblotting with Ab4. Immune complexes were visualized with luminol.

Synthesis and activity of Ad12 DNApol during abortive infection

We have previously shown that both Ad12 DNApol and pTP are synthesized in sufficient quantities during abortive infection to catalyze in vitro the initiation reaction of Ad12 DNA replication (Chowrira and Lucher, 1990). Incorporation of [3 H]TTP or [γ - 32 P]TTP into poly(dA):oligo(dT) was used to assay the activity of Ad12 DNApol in a manner presumably independent of pTP activity. Because the specificity of dA:dT for Ad DNApol was previously shown using purified Ad5 protein (Fields et al., 1984), we optimized assay conditions for crude cytoplasmic and nuclear extracts from infected, hydroxyurea-treated cells (Fig. 4A). Under the optimized conditions of 1 hr incubation with 4 nmol dA:dT, there was no significant difference in DNApol activity in KB and BHK extracts (Fig. 4B). We previously showed that the initiation activity of either cytoplasmic or nuclear extracts from

infected BHK cells is two- to five-fold lower than from infected KB cells, but that this is most likely due to reduced efficiency of BHK nuclear factors in the reaction rather than reduced levels of DNAPol:pTP complex (Chowrira and Lucher, 1990).

Figure 4. Ad12 DNAPol in infected KB and BHK extracts. All extracts were prepared from cells infected or mock infected for 48 hr in the presence of hydroxyurea. (A) Optimal concentration of dA:dT to assay DNAPol activity. Reaction mixtures contained 1 nmol (closed circles) or 4 nmol (open triangles) of dA:dT and 20 μ g protein from nuclear extracts of infected or mock-infected KB cells. The left panel shows the raw values for both infected (I, closed circles and open triangles) and mock-infected (M, closed triangles and open boxes) extracts, while the right panel shows the net values for infected extracts. (B) Comparison of DNAPol activity in infected KB and BHK extracts. Reaction mixtures containing 4 nmol dA:dT and 20 μ g protein from cytoplasmic (CE) or nuclear (NE) extracts were incubated for 1 hr. Activities shown are net values for infected extracts and are the average of three determinations. Standard error of the mean is shown for each. (C) Detection of Ad12 DNAPol using anti-peptide antiserum (Ab6). Aliquots of cytoplasmic extract (60 μ g protein) from infected and mock-infected KB and BHK cells were analyzed by nondenaturing PAGE on a 7% gel, followed by immunoblotting with Ab6 in the absence ($\alpha 6$) or presence ($\alpha 6 + P$) of competing homologous peptide. Immune complexes were visualized with 4-chloro-1-naphthol. The hatch mark to the left indicates the position of the 116K prestained marker protein; DNAPol is indicated by the bracket. The specificity of Ab6 for Ad12 DNAPol from infected KB extracts has been shown elsewhere (Chowrira et al. 1991).

To compare the accumulation levels of Ad12 DNAPol with the activity levels, immunoblot analysis with antipeptide 6 antiserum (Ab6) was performed on extracts of KB and BHK cells infected in the presence of hydroxyurea. The protein was readily detectable in cytoplasmic extracts of both KB and BHK cells and accumulated to approximately equal levels (Fig. 4C). However, DNAPol was not detectable with Ab6 in either KB or BHK nuclear extracts (not shown). It is possible that alterations in the target epitope in nuclear DNAPol prevent Ab6 from binding in the immunoblots.

Effect of Ad5 E1 region on Ad12 DBP synthesis in hamster cells

Expression of the Ad2 or Ad5 E1 region during Ad12 infection of hamster cells allows replication of Ad12 DNA (Klimkait and Doerfler, 1985, 1987). We therefore sought evidence for a corresponding effect on the expression of Ad12 DBP during such complementation. To this end we tested an Ad5 E1-transformed hamster cell line for complementation of Ad12 DNA replication. The 983.2 cell line expresses the Ad5 E1A, E1B 496R (58K), and E1B 175R (19K) proteins (Rowe et al., 1984). Ad12 infection of 983.2 cells resulted in a modest level of Ad12 DNA replication (Fig. 5A); by 72 hr p.i., a 10- to 12-fold increase in intact Ad12 DNA over parental viral DNA was observed by Southern analysis. Degradation of viral DNA was also evident at both 48 and 72 hr p.i. (Fig. 5A). There was no detectable increase in parental Ad12 DNA in BHK cells infected under the same conditions (Fig. 5A).

We confirmed the identity of replicated DNA as Ad 12 by restriction digestion with each of four different enzymes followed by Southern analysis (Fig. 5B). When probed with the Ad12 E4-containing plasmid gE4-2, a single band of the following approximate sizes was detected for each digestion: *Sma*I, 15.14 kb; *Kpn*I, 5.01 kb; *Bam*HI, 4.27 kb; and *Hind*III, 3.80 kb. The corresponding predicted sizes for these bands (based on map unit locations of the restriction sites; Tooze, 1980) are approximately 15.10, 5.22, 4.34, and 3.40 kb, respectively.

Immunoblot analysis of whole cell extracts from infected 983.2 cells showed a dramatic effect on DBP concentration. At equal m.o.i., DBP accumulation was approximately 120-fold greater in 983.2 than in BHK cells (Fig. 5C). Because DNA replication occurring in 983.2 cells could have influenced the concentration of DBP, synthesis was also analyzed in extracts of hydroxyurea-treated cells. Hydroxyurea did not affect the accumulation of DBP in 983.2 cells, and DBP in both cytoplasmic and nuclear extracts of hydroxyurea-treated 983.2 was roughly equivalent to that seen in extracts of similarly treated KB cells at 1/3 m.o.i. (Fig. 5D). The activity of Ad12 DNAPol was not significantly different in 983.2 and KB cell extracts (not shown).

Figure 5. Effects of Ad5 E1 expression on Ad12 infection of hamster cells. (A) Complementation of defective Ad12 DNA replication during infection of 983.2 cells. Nine hundred and fifty nanograms of DNA from infected and mock-infected 983.2 and BHK cells was electrophoresed in 1% agarose and analyzed by Southern blotting with plasmid gE4-2. DNA was extracted at 2, 48, and 72 hr p.i.; DNA was extracted from mock-infected (M) cells at 72 hr p.i. Ad12 DNA is indicated by the arrowhead; hatch marks show the positions of the 23.13 and 0.56 kb bands from *Hind*III-digested λ DNA used as markers. (B) Mobilities of products detected after restriction digestion of replicated Ad12 DNA. DNA isolated from infected 983.2 cells at 72 hr p.i. was cut with *Sma*I (S), *Kpn*I (K), *Bam*HI (B), or *Hind*III (H) and analyzed by Southern blotting with plasmid gE4-2. Closed squares, *Hind*III-digested λ marker DNA fragments; closed circles, fragments detected with plasmid gE4-2. (C) DBP synthesis during infection of 983.2 cells. Aliquots containing 60 μ g protein from 983.2, or 120 μ g protein from BHK cells infected for 48 hr with the indicated m.o.i. were analyzed by immunoblotting with Ab4. Immune complexes were visualized with luminol. The BHK panel is the same as in figure 2A. (D) Distribution of DBP in infected 983.2 cells. KB and 983.2 cells were infected (m.o.i. of 897 and 2690, respectively) or mock-infected for 48 hr in the presence of hydroxyurea, and cells were fractionated by the Nonidet P-40/NaCl method. Forty micrograms of protein from cytoplasmic (C) or nuclear (N) extracts were analyzed by immunoblotting with Ab4; immune complexes were visualized with luminol. In the right-hand panel, nuclear extracts were prepared from 983.2 cells infected in the absence (-HU) or presence (+HU) of hydroxyurea.

Analysis of another early protein, the Ad12 E1B 163R, showed that the effect of the 983.2 cell environment on Ad12 protein expression was not limited to the DBP. Immunoblots performed with antipeptide 8 antiserum (Ab8) did not detect the Ad2 E1B 175R protein (Fig. 6A), indicating that the antiserum would not react with the Ad5 175R protein expected in extracts of 983.2 cells. Since viral DNA replication increased the accumulation of 163R during productive infection (Fig. 6B), extracts from KB, BHK, and 983.2 cells infected in the presence of hydroxyurea were analyzed for 163R protein. Accumulation of the E1B 163R protein was equivalent in KB and 983.2 cells in the presence of hydroxyurea (Fig. 6C).

Figure 6. Accumulation of the Ad12 E1B 163R protein in 983.2 cells. In all cases, cell extracts were analyzed by immunoblotting with Ab8, and immune complexes were visualized with luminol. (A) Specificity of Ab8 for the 163R protein from Ad12. Crude extracts (40 μ g protein) of KB cells infected for 48 hr with Ad12, or for 24 hr with Ad2, were analyzed by immunoblotting. Mock-infected extract (M) was included as negative control. The arrowhead indicates the 163R protein; the hatch mark shows the position of the 26K prestained molecular weight marker. (B) Effect of hydroxyurea on the accumulation of 163R protein during infection of KB cells. Crude extracts (40 μ g protein) of KB cells infected at the indicated m.o.i. for 48 hr in the absence (-HU) or presence (+HU) of hydroxyurea were analyzed. (C) Effect of Ad5 E1 present in 983.2 cells on 163R accumulation. KB, BHK, and 983.2 cells were infected for 48 hr in the presence of hydroxyurea and were fractionated into cytoplasmic (C) and nuclear (N) extracts by the Nonidet P-40/NaCl method. Aliquots containing 40 μ g of KB or 983.2 protein, or 120 μ g of BHK protein, were analyzed.

Discussion

We have analyzed the expression of Ad12 E2 proteins during abortive infection of BHK cells. Use of hydroxyurea to block the formation of endogenous replication complexes (Challberg and Kelly, 1979) not only allowed us to detect Ad 12 DNApol activity in extracts from infected cells but also allowed us to compare DBP concentrations in the absence of DNA replication in KB and 983.2 cells. Our data indicate that Ad12 DBP is drastically reduced during abortive infection; thus DBP concentration may be too low to support DNA replication *in vivo*, just as E1A activities *in vivo* are dependent on sufficient E1A concentrations (Vaessen et al., 1987). Although DBP synthesized during abortive infection binds to denatured DNA-cellulose, its elution at a lower salt concentration suggests that it may not bind to DNA efficiently. However, it is also possible that the larger concentration of BHK proteins applied to the column effectively competed with the low amount of DBP for binding to DNA. DBP synthesized during abortive infection can be found in the nucleus, so no major transport defect was detected by our cell fractionation analyses.

Ad12 DNApol from infected BHK cells is active in two *in vitro* assays. The results with dA:dT show DNApol activity presumably independent of pTP, and without significant interference from cellular polymerases. The equivalent activity in KB and BHK extracts using dA:dT is consistent with our previous analysis of the *in vitro* initiation reaction (Chowrira and Lucher, 1990). Since DNApol activity with dA:dT is not altered during infection of 983.2 cells, the expression of the Ad12 E2B genes appears to be unaffected by these cellular environments under our infection conditions, unlike other early genes. Although active pTP must be synthesized during abortive infection to support initiation *in vitro*, we lacked reagents to directly determine the concentration of pTP in this study.

DNApol accumulates to equivalent levels in cytoplasmic extract from both KB and BHK cells, which correlates with equivalent activity in these extracts for initiation in the presence of KB nuclear factors (Chowrira and Lucher, 1990), and deoxynucleotide incorporation into product from dA:dT template. We expected a similar correlation between DNApol activity and concentration in KB and BHK nuclear extracts, but we were unable to directly determine nuclear concentration with Ab6. Synthetic peptide antigens must assume the same or a very similar conformation as the target sequence in the intact protein if the antibodies are to bind to the protein of interest (discussed in Berzofsky, 1985). The conformation of the epitope targeted by Ab6 must be critical, since even cytoplasmic DNApol could not be detected by immunoblotting after denaturation and SDS-PAGE (not shown). It is therefore possible that nuclear Ad 12 DNApol in both productive and abortive infection exhibits an altered conformation of the target epitope for Ab6, such that it cannot be bound by the antiserum.

The importance of viral gene products other than the replication proteins to the mechanism of abortive infection is indicated by the ability of the E1 region of group C Ads (e.g., Ad2 or Ad5) to complement the Ad12 DNA replication defect (Klimkait and Doerfler, 1985, 1987). This complementation phenomenon is exhibited by the 983.2 cell line used in this study, but degradation of replicated viral DNA is evident in these cells. The Ad5 E1B 175R/Ad12 E1B 163R protein is responsible for protecting viral DNA from degradation during infection (Grand, 1987), so the Ad12 163R protein may not function efficiently in

983.2 cells in spite of its higher concentration compared to abortive infection. The concentration and/or activity of endogenous Ad5 175R protein in 983.2 cells may also be insufficient to protect replicating Ad12 DNA.

Our results suggest that Ad2/5 E1 complementation of Ad12 DNA replication in hamster cells is due in part to stimulation of DBP concentration. The complementation effect is probably a broadly acting phenomenon, since we also observe an increase in E1B 19K accumulation in extracts from these cells. Approximately five-fold more input Ad12 DNA can be extracted at 2 hr p.i. from infected 983.2 cells than from BHK cells, so it appears that more virus is able to enter 983.2 cells at equal m.o.i. This increase in the efficiency of Ad12 entry may not be directly related to Ad5 E1 activities. However, the 120-fold increase in DBP accumulation in 983.2 over BHK cells cannot be accounted for solely by an increase in input viral DNA. In addition, the DNA extraction procedure cannot determine the percentage of input viral DNA that is uncoated and expressing viral genes. The mechanisms of E1 complementation may therefore include effects at the level of transcription and/or translation of early Ad12 genes.

The simplest mechanism of control over early gene expression during abortive infection would operate at the transcriptional level. The hypothesis we are currently testing is that reduced concentrations of E1A lead to reduced early gene activation, and hence reduced early protein levels. The lower concentrations of E1B and E2A proteins during abortive infection are consistent with this hypothesis; however, E2B proteins appear to be unaffected by the hamster environment under our experimental conditions. The E2 transcription unit in Ad5 is controlled by two promoters, one at 75 m.u. (map units), which is active at early times postinfection, and one at 72 m.u., which is activated at intermediate times postinfection (reviewed in Persson and Philipson, 1982). The DBP gene is expressed from each promoter, whereas DNAPol and pTP are expressed only from the 75 m.u. promoter. If the Ad12 E2 unit is similar to the Ad5, it is possible that the 72-m.u. promoter is defective in hamster cells. This could reduce DBP expression without necessarily affecting expression of DNAPol or pTP. Others have shown that the Ad12 major late promoter does not function in hamster cells (Weyer and Doerfler, 1985).

In summary, we have shown that the Ad12 DBP is synthesized during abortive infection of BHK cells by Ad12, but at a greatly reduced level when compared to that of productive infection. The DBP concentration is affected by Ad5 E1 genes under conditions that allow Ad12 DNA replication in hamster cells, suggesting that a reduction in DBP plays a role in abortive infection.

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