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Adeno-Associated Virus Helper Activity of Adenovirus DNA Binding Protein

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The requirement for the adenovirus (Ad) single-stranded DNA binding protein (DBP) in the expression of adeno-associated virus (AAV) proteins was studied by specific immunofluorescent staining of infected cells and in vitro translation of RNA from infected cells. The Ad5 mutant ts125, which carries a mutation in the DBP gene, helped AAV as efficiently as the Ad5 wild type (WT) did at both the permissive (32°C) and nonpermissive (40.5°C) temperatures in HeLa and KB cells. Furthermore, at 40.5°C ts125 was as efficient as Ad5WT was in inducing the expression of AAV proteins in a line of Detroit 6 cells which is latently infected with AAV. However, little if any AAV protein was synthesized when coinfections were carried out with Ad5WT in CV-C cells, a monkey cell line that is highly restrictive for human Ad replication unless the cells are also infected with simian virus 40. On the other hand, AAV protein was efficiently produced in CV-C cells in coinfections with the Ad5 mutant hr404, whose growth is unrestricted in CV-C cells and whose mutation also maps in the DBP gene. Finally, preparations of cytoplasmic RNA extracted from CV-C cells infected with AAV and Ad5WT or from CV-C cells infected with AAV, Ad5WT, and simian virus 40 were each capable of directing the in vitro synthesis of abundant amounts of AAV proteins in a rabbit reticulocyte lysate system. These results indicate that the abnormal DBP of ts125 still retains its helper function for AAV replication, but that the molecular feature of the DBP which relates to the monkey cell host range restriction of Ad's may also account for the observed block to AAV protein translation in CV-C cells.

Adeno-associated viruses (AAV) are defective parvoviruses whose replication requires coinfection with a helper adenovirus (Ad) (28) or herpesvirus (4). Without a helper virus coinfection, there is no detectable synthesis of AAV DNA, RNA, or protein. Janik et al. (13) have mapped the specific Ad genes necessary for AAV replication. They are early Ad genes located in regions E1a, E2a, and E4 and in the region encoding VA I RNA. A region E1a product may be necessary for the efficient transcription of region E2a (15), and interactions may also occur among gene products from regions E2a and E4 and VA I RNA (13, 24). The sole gene product of region E2a is the Ad single-stranded DNA binding protein (DBP) (34), which forms large intranuclear particulate inclusions in infected cells (33). To further define the means by which the Ad DBP facilitates AAV growth, we have examined AAV replication under conditions in which Ad production is impaired by alterations in DBP activity (i.e., temperature-sensitive mutation and host range restriction in monkey cells). It was found that AAV type 2 (AAV2)-specific nuclear inclusions are distinct from Ad DBP inclusions and that the immunofluorescent staining patterns for Ad DBP are altered by AAV coinfection. Furthermore, large DBP inclusions do not form at the nonpermissive temperature with ts125 (a DNA-minus, temperature-sensitive mutant of Ad5 that carries a mutation within the DBP gene [8, 22]) or with ts149 (a DNA-minus, temperature-sensitive mutant of Ad5 that carries a mutation within region E2b [31]). Additionally, contrary to findings reported by others (14, 23), we have established that ts125 is as efficient as Ad5 wild type (WT) in supporting AAV protein synthesis and replication. Finally, we show that the Ad5 host range restriction in a continuous line of monkey cells (CV-C cells) also is associated with a block to AAV protein translation in vivo, although abundant AAV mRNA is present in the cytoplasm and is readily translated in vitro.

MATERIALS AND METHODS

Cells and medium. KB cells were grown in Eagle medium either in spinner cultures supplemented with 5% horse serum or in monolayers supplemented with 10% fetal calf serum. HeLa cells (originally from J. Williams), CV-C cells (a continuous line of African green monkey kidney cells derived from CV-1 cells [1], obtained from W. Richardson), and Detroit 6 cells latently infected with AAV1 (provided by M. D. Hoggan) were grown in monolayers in Eagle medium supplemented with 10% fetal calf serum.

Virus stocks. Ad5WT and the host range mutants Ad5 hr404 and Ad2 hr400 (obtained from D. Klessig) were propagated in KB cells in spinner culture for 40 h at 37°C. The infected cells were concentrated 50× in fresh medium, frozen and thawed four times, clarified of cellular debris by brief centrifugation, and stored at −70°C. AAV was produced by coinfection with Ad5 in KB cells which were pelleted 40 h after infection. The cells were suspended in 0.05 M Tris buffer, pH 8.0, frozen and thawed, and sonicated, and the AAV virions were banded in a cesium chloride gradient (32). Virion bands were diluted 10-fold with 0.01 M Tris (pH 8.1)–0.15 M NaCl–0.1% bovine serum albumin–50% glycerol (25) and heated at 56°C for 10 min to inactivate any contaminating Ad. This AAV stock was distributed into vials and stored at −20°C until use. The Ad5 stock contained no AAV contaminant and the AAV stock contained no Ad contaminant as judged by immunofluorescent staining of HeLa cell monolayers infected with either stock alone. Seed stocks of ts125 and ts149 were propagated in KB cells for 72 h at 32°C and concentrated as described above. Titers of Ad5WT, ts125, and ts149 were obtained by plaquing on HeLa cell monolayers at 32°C (37). AAV was titrated as fluorescent focus-forming units (FFU) at 28 h after coinfection with Ad5WT in HeLa cell monolayers grown at 37°C.

Infections and immunofluorescence. Trypsinized cells (HeLa, CV-C, and Detroit 6) were plated out in 60-mm-diameter plastic dishes containing one or two glass cover slips (11 by 22 mm). The next day, monolayers were washed with medium containing 1% agammaglobulinic calf serum. Adsorption of infecting virus was carried out for 1 h in a volume of 0.5 ml of the appropriate virus dilution in 1% agammaglobulinic calf serum. Cells were then washed with 2 ml of 1% agammaglobulinic calf serum and fed with medium containing 10% fetal calf serum. The infected cells were incubated at 32, 37, or 40.5°C until they were harvested. Cover slips were then quickly washed twice in cold phosphate-buffered saline, fixed in acetone at −20°C, cut into halves, and stained by an indirect immunofluorescence technique. The sera used were rabbit anti-DBP, guinea pig anti-AAV1 virion, guinea pig anti-AAV2 virion, and guinea pig anti-Ad2 virion. The second antibody was either fluorescein-conjugated goat anti-guinea pig gamma globulin, fluorescein-conjugated goat anti-rabbit gamma globulin, or rhodamine-conjugated goat anti-guinea pig gamma globulin. All fluorescent-conjugated antisera were purchased from Cappel Laboratories. Photomicroscopy was performed with a Zeiss Fluorescent Photomicroscope II and Kodak ASA 400 black and white print film. From 400 to 1,000 cells were counted for each infection to obtain percentages of cells positive for particular antigens.

In vitro translation and electrophoresis. Infected CV-C cell monolayers in 150-cm^2 plastic tissue culture flasks were removed by brief trypsinization, mixed with medium containing 10% fetal calf serum, centrifuged, and washed with cold phosphate-buffered saline. After treatment with hypotonic buffer for 15 min, cells were disrupted by 20 strokes in a Dounce homogenizer, and the nuclei were removed by centrifugation (7). Cytoplasmic RNA was obtained from corresponding supernatants by being pelleted in Sarkosyl-cesium chloride (11). After three precipitations in ethanol, RNA samples were dissolved in sterile distilled water and translated in vitro in a rabbit reticulocyte lysate system with [^{35}S]methionine (Amersham Corp.) (6). Each translation mixture was programmed with the RNA from about 10^5 cells. The products were analyzed by sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis (5, 21). For autofluorography, gels were fixed, soaked in En^{3}Hance (New England Nuclear Corp.), dried onto 3MM filter paper, and exposed to prefogged X-ray film at −70°C.

RESULTS

Immunofluorescent staining patterns of Ad5WT DBP. The characteristic intranuclear inclusions of Ad DBP (Fig. 1A) were distinct and separate from the AAV-positive inclusions (Fig. 1B) in HeLa cells coinfected with Ad5WT and AAV2. Although these particular cells contained both AAV inclusions and DBP inclusions, it should be emphasized that in most coinfected cells the evolution of a globular deposition of Ad DBP was almost completely inhibited in the nuclei of cells that contained AAV inclusions (Fig. 1). In these cells, the DBP deposited more uniformly in the nuclei but spared regions which contained intensely staining inclusions of AAV. Typically, the bulk of the nucleus in a coinfected cell was diffusely positive for both Ad DBP and AAV in a completely overlapping distribution.

DBP patterns with ts125 and ts149. The globular accumulation of Ad DBP seen with Ad5WT (Fig. 2A) did not occur at the nonpermissive temperature with ts125, a DNA-minus mutant that synthesizes a thermolabile DBP (35) owing to a point mutation in the DBP gene (20). Instead, lightly staining amounts of the ts125 DBP localized in the nuclei of infected cells, leaving nonstaining regions which appeared to include normal nuclear structures such as nucleoli (Fig. 2C). However, ts125 clearly induced AAV protein synthesis (Fig. 2D). Another DNA-minus, temperature-sensitive Ad mutant, ts149, carries a mutation which maps away from the DBP gene into region E2b, which codes for the Ad terminal protein (31) and probably a DNA-polymerase (B. Stillman, personal communication). At the nonpermissive temperature this mutant produced an abundant amount of normal DBP, but, as with ts125, accumulation also occurred in a

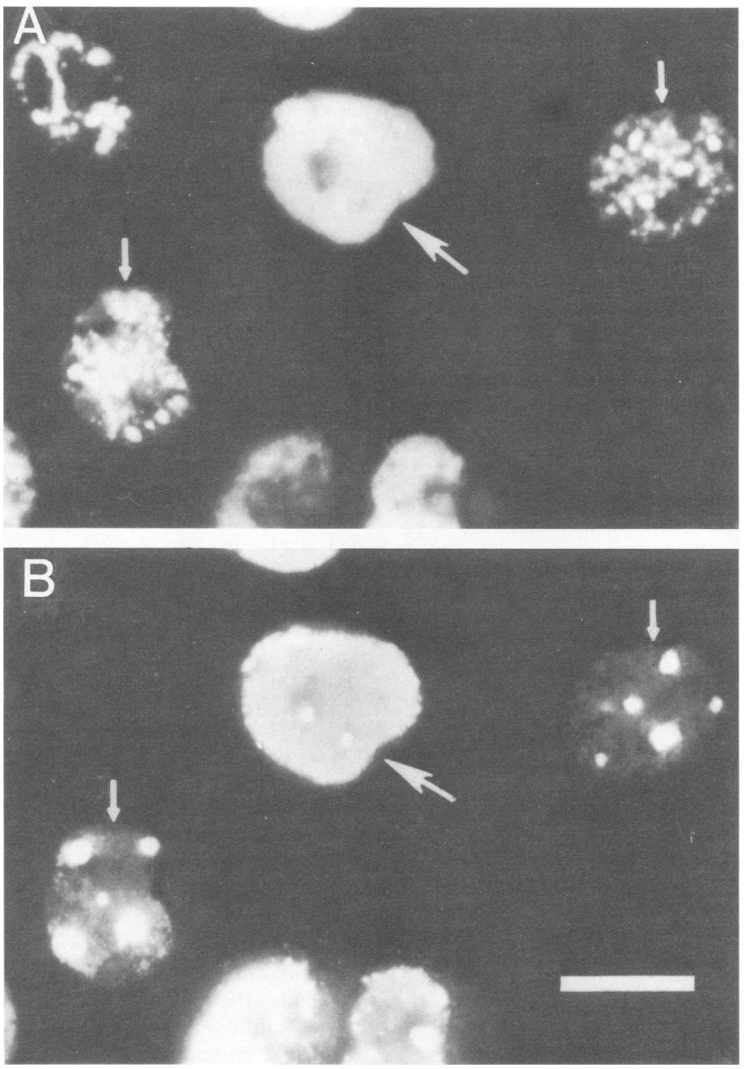

FIG. 1. Comparison of the immunofluorescent staining patterns of Ad DBP and AAV virion proteins in HeLa cells. The same cells were stained for Ad DBP with a fluorescein-conjugated anti-globulin (A) and for AAV protein with a rhodamine-conjugated anti-globulin (B). The center cell (large arrow), whose nucleus is diffusely positive for both DBP and AAV (except for intensely staining AAV-positive inclusions), was typical of most coinfected cells. Occasional cells (small arrows) had distinct inclusions of DBP or AAV. Some cells (e.g., cell in upper left corner of A) had the characteristic globule formation of Ad DBP without expressing AAV (cell not appearing in B). Bar, 20 μm.

nonglobular distribution (data not shown). Occasionally, however, ts149-infected cells produced globular deposits of DBP at the nonpermissive temperature, but such cells were infrequent and their DBP inclusions were less well formed than those seen in parallel Ad5WT infections. As occurred in AAV coinfection with ts125, ts149 efficiently induced AAV protein synthesis at the restrictive temperature. As expected, at the permissive temperature ts125 and ts149 produced DBP inclusions similar to those seen with Ad5WT, and they also complemented one another for WT DBP inclusion formation at the nonpermissive temperature.

Quantitation of AAV2 helper activity. HeLa cell monolayers on cover slips were infected with Ad5WT, ts125, ts149, or ts125 plus ts149. After adsorption of the virus for 1 h, duplicate cultures were either additionally infected with AAV2 or mock infected and then incubated at 32°C for 40 h or at 40.5°C for 18 h. Cover slips were then fixed and stained for late Ad virion

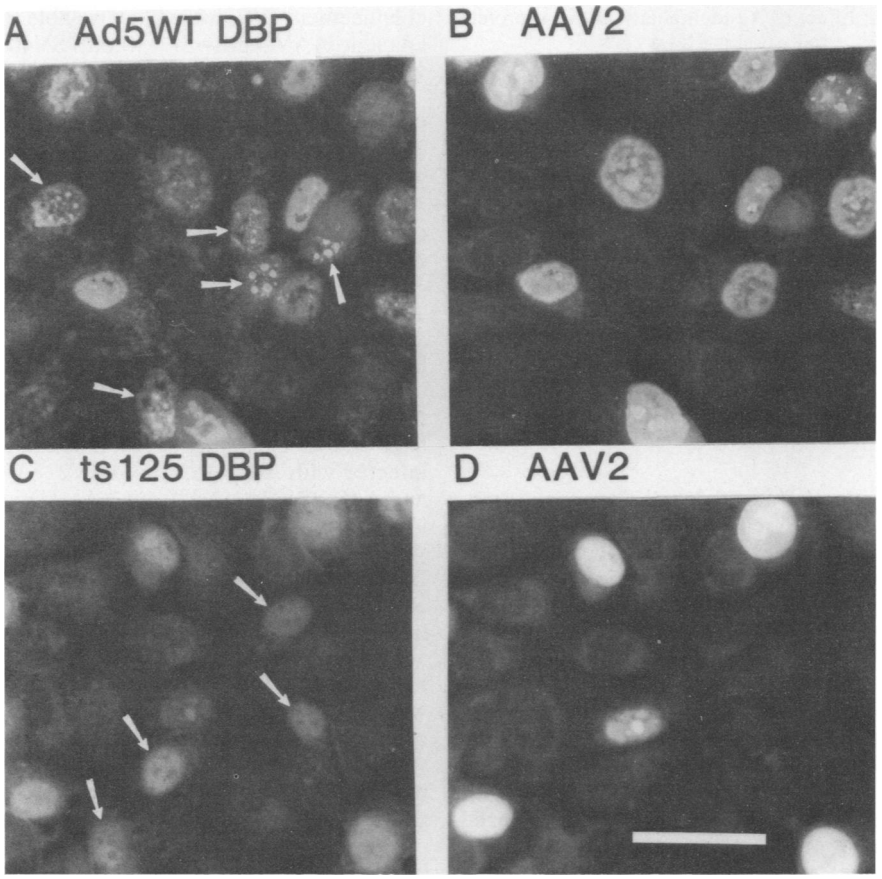

FIG. 2. AAV positive immunofluorescence with Ad5WT or ts125 used as helper virus at 40.5°C. The immunofluorescent technique used for DBP (A and C) and AAV (B and D) is described in the legend to Fig. 1. Arrows indicate cells expressing DBP but no AAV. Ad5WT infection shows characteristic DBP globules (A), whereas ts125 infection shows only faint diffuse staining for DBP (C) at this nonpermissive temperature. AAV protein accumulation did occur when Ad5WT (B, same field of cells as in A) or ts125 (D, same field of cells as in C) was the helper virus. The faint inclusions in cells infected with AAV and ts125 in C actually arise from rhodamine fluorescence specific for AAV (D) and do not represent true DBP globules. Bar, 50 μm.

proteins or AAV2 protein to measure Ad and AAV infectivity and complementation between ts125 and ts149. There was no substantial difference in the ability of Ad5WT, ts125, or ts149 to help AAV2 replication at permissive or nonpermissive temperatures, despite complete inhibition of Ad late protein synthesis at the nonpermissive temperature with either ts125 or ts149 (Table 1). It should be noted that coinfection with ts125 and ts149 at 40.5°C yielded late Ad proteins and thus verified the expected complementation between these mutants. When AAV and serial dilutions of Ad5WT or ts125 were used to coinfect HeLa cell monolayers, there was a comparable reduction in the percent of cells positive for AAV2 at permissive and non-

TABLE 1. Expression of AAV and Ad proteins in HeLa cells

Helper Ad[a]	% of cells fluorescent for:			
	AAV2 protein[b]		Late Ad protein[c]	
	32°C	40.5°C	32°C	40.5°C
Ad5WT	33.9	64.3	32.1	69.7
ts125	34.0	43.0	31.4	0
ts149	18.0	48.0	17.2	0
ts125 + ts149	32.4	57.2	40.1	25.6
None	0	0	0	0

[a] Multiplicity of infection was approximately 10 PFU per cell for each helper Ad.

[b] Cells were coinfected with AAV2 at a multiplicity of 10 FFU per cell.

[c] Cells were not coinfected with AAV2.

TABLE 2. Effect of Ad multiplicity on AAV protein production in HeLa cells[a]

Helper Ad	Helper Ad multiplicity (PFU per cell)	% of cells grown at indicated temp (°C) fluorescent for AAV2 protein	
		32	40.5
Ad5WT	6.0	21.6	40.3
	1.2	7.1	9.8
	0.24	2.0	2.3
ts125	2.0	24.6	26.5
	0.4	7.4	8.0
	0.08	1.4	2.6
None	0	0	0

[a] AAV2 multiplicity was 10 FFU per cell.

permissive temperatures which was proportional to the dilution of either helper virus (Table 2). This reduction even occurred at Ad multiplicities below 1 PFU per cell, indicating that single-hit infections of ts125 or Ad5WT are equally capable of initiating AAV replication.

Rescue of latent AAV1. Monolayers of Detroit 6 cells latently infected with AAV1 (i.e., they do not produce detectable AAV1 antigens unless infected with a helper Ad [3]) were grown on cover slips, infected with Ad5WT, ts125, or ts149, and then stained for AAV1 or late Ad proteins. Although ts125 and ts149 synthesized no late Ad proteins at the nonpermissive temperature, they were as effective as Ad5WT was in inducing AAV1 protein synthesis (Table 3).

DBP patterns in CV-C cells. CV-C cells are a continuous line of monkey kidney cells that is highly restrictive for human Ad replication (1). Little or no stainable Ad late proteins are made unless the cells are also infected with simian virus 40 (SV40). When CV-C cells were infected with Ad5 alone or with SV40 plus Ad5, the pattern of staining for the DBP was similar to that found in Ad5-infected HeLa cells (Fig. 1A). The overall amount of DBP accumulated appeared to be the same in human and monkey cells, as judged by the brilliance of immunofluorescence and the number of positive cells. In CV-C cells coinfected with Ad5 and AAV2, only an occasional cell (about 1 in 1,000) was positive for small, sparse inclusions staining for AAV2 virion proteins. However, CV-C cells triply infected with SV40, Ad5, and AAV2 were virtually all strongly positive for stainable AAV2 protein. A comparable helper response was obtained in CV-C cells when ts125 was used in place of the Ad5WT. SV40 alone was unable to help the expression of AAV2 proteins. In addition, we found that both hr404 and hr400, host range mutants of Ad5 and Ad2, respectively, which replicate in monkey cells without SV40 enhancement (19), were likewise able to support efficient AAV2 growth without SV40 coinfection.

AAV2 infection afforded the same inhibition to Ad DBP globule formation in CV-C cells as it did in HeLa cells (Fig. 1A and B). This inhibition was the same in CV-C cells infected with SV40, Ad5, and AAV2 (in which case AAV2 virion proteins were expressed) as it was in CV-C cells infected with Ad5 and AAV2 (in which case AAV2 virion proteins were not expressed). Thus, the alteration in aggregation of Ad DBP apparently relates to AAV DNA or RNA synthesis but not to AAV protein synthesis.

In vitro translation of AAV RNA synthesized in CV-C cells. Cytoplasmic RNA was prepared from CV-C cells that were mock infected or infected with Ad5, Ad5 and AAV2, SV40, SV40 and Ad5, or SV40, Ad5, and AAV2 (Fig. 3). The in vitro translation of these RNA preparations yielded abundant amounts of AAV2 proteins A, B, and C (30) independent of coinfection with SV40 (Fig. 3, lanes 3 and 6). This suggests a block to AAV2 protein synthesis in vivo directly at the translational level. Translatable messages for Ad5 hexon (protein II) and fiber (protein IV) were more abundant in the CV-C cells coinfected with SV40 (lane 5) than in those cells infected with Ad5 alone (lane 2). Hence, the host range block to AAV2 protein synthesis differed from the block to Ad protein synthesis in that SV40 coinfection enhanced the levels of certain translatable Ad messages. Finally, it can be seen that AAV2 coinfection diminished the amount of translatable Ad5 RNA from an infection enhanced by SV40 (lane 6), again demonstrating a competitive or suppressive interaction exerted by AAV on Ad replication.

Yield of infectious AAV2 in KB cells. It has been recently reported that at the nonpermissive temperature ts125 is an inefficient AAV helper in KB cells (14, 23). Because this conflicts with previous data (32), we reexamined the ability of ts125 to help AAV replication in KB cells by

TABLE 3. Induction of AAV1 protein synthesis in latently infected Detroit 6 cells

Helper Ad	Helper Ad multiplicity (PFU per cell)	% of cells fluorescent for:			
		AAV1 protein		Late Ad protein	
		32°C	40.5°C	32°C	40.5°C
Ad5WT	11	15.3	32.7	84.2	62.9
	2.2	10.5	35.0	66.9	30.8
ts125	40	18.6	32.4	91.1	0
	8	16.2	20.5	73.1	0
ts149	40	18.1	41.2	80.8	0
	8	11.5	21.2	49.5	0
None	0	0	0	0	0

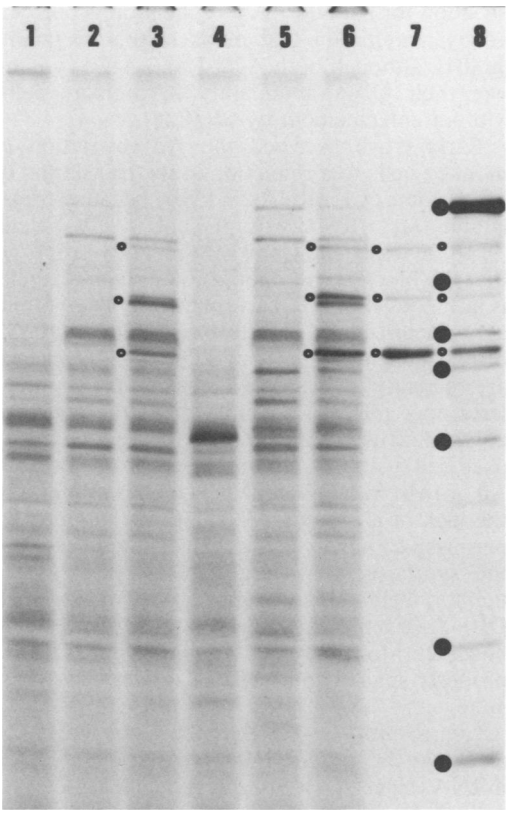

FIG. 3. [^{35}S]methionine-labeled products of in vitro translation programmed with RNA obtained from CV-C cells. Translations of RNA from uninfected cells (lane 1) and from cells infected with Ad5 (lane 2), Ad5 and AAV2 (lane 3), SV40 (lane 4), SV40 and Ad5 (lane 5), and SV40, Ad5, and AAV2 (lane 6). Lane 7, Purified AAV virion ^{35}S-labeled proteins; lane 8, mixture of purified Ad5 and AAV virion ^{35}S-labeled proteins. The small circles at the left of some lanes indicate AAV proteins A, B, and C (from top to bottom). The large circles indicate Ad5 virion proteins II, III, IIIa, IV, V, VI, and VII (from top to bottom) in lane 8.

measuring yields of infectious AAV virions after coinfections at the permissive and nonpermissive temperatures.

KB monolayers in 60-mm plastic dishes were infected with Ad5WT, ts125, ts149, ts125 and ts149, or with no helper Ad. After a 1-h adsorption period, the monolayers were washed, infected with AAV2, and incubated at 32°C for 40 h or at 40.5°C for 24 h. The infected monolayers were harvested by being scraped into the culture medium (5 ml). They were freeze-thawed four times, heated at 56°C for 10 min, and clarified of cellular debris by brief centrifugation. The titer of infectious AAV2 in each supernatant was determined by immunofluorescent staining of HeLa cell monolayers coinfected with serial dilutions of the supernatants and a constant multiplicity (5 PFU per cell) of Ad5WT at 37°C. These titers were then divided by the number of cells in the originally infected KB cell monolayers to obtain the burst size (FFU per cell). There was no substantial difference between the AAV yields produced with the WT or with either ts mutant at the nonpermissive or permissive temperature (Table 4). Similar results were obtained in two additional experiments. Thus, ts125 efficiently helped AAV replication in KB cells at the restrictive temperature.

DISCUSSION

The analysis of AAV replication in permissive and nonpermissive cells with certain Ad mutants as helpers has provided additional insight into possible regulatory features of the Ad DBP. It is well established that at the restrictive temperature ts125, although DNA-minus for Ad, efficiently promotes AAV DNA synthesis (23, 32). Furthermore, Straus et al. (32) found that ts125 effectively supports AAV antigen production. Subsequently, Myers et al. (23) reported that accumulation of AAV protein and infectious virions is deficient when KB cells are coinfected with ts125 at the nonpermissive temperature. They proposed that this deficiency is due to impaired AAV mRNA translation which, in turn, is attributed to the defective DBP of ts125 (14). Our present results do not support this conclusion (Tables 1 through 4). To rule out the possibility that another strain of ts125 was deficient in helping AAV, we obtained another ts125 stock (14, 23) and found it fully capable of supporting AAV replication at the nonpermissive temperature. Furthermore, complementation of this virus with ts149 also yielded late Ad proteins. Efficient support of AAV replication by ts149 confirms that a product of region E2b which is needed for Ad DNA replication (31) is not required by AAV (23, 32).

AAV replication undoubtedly proceeds at the

TABLE 4. Yields of infectious AAV2 from KB cells grown in monolayers[a]

Helper Ad	AAV yield per cell (FFU)	
	40.5°C	32°C
Ad5WT	6,832	5,634
ts125	3,696	5,656
ts149	6,922	5,723
None	0.18[b]	0.07[b]

[a] Multiplicities of virus infection: 10 PFU per cell for Ad5WT, ts125, and ts149; 10 FFU per cell for AAV2.

[b] Residual AAV2 infectivity.

expense of its helper Ad. For example, a decreased accumulation of late Ad proteins in coinfected cells (4) could result from a decrease in the amount of translatable Ad mRNA (Fig. 3, lanes 5 and 6) due to a diminished number of transcriptional templates secondary to inhibition of Ad DNA synthesis (4, 29). In the present study, AAV interference with Ad DNA synthesis is also suggested by the immunofluorescent staining pattern of Ad DBP in AAV coinfections (Fig. 1 and 2). In cells staining brightly for AAV-positive inclusions, the normal development of DBP inclusions was largely prevented, and the deposition of DBP was diffuse in the nucleus. This pattern of DBP staining was similar to that observed when Ad DNA synthesis is known to be deficient (e.g., infections with ts125 or ts149 at the nonpermissive temperature [Fig. 2C] and cytosine arabinoside-treated AdWT infection [33]). One consequence of inhibition of Ad DNA synthesis might be a delay in the shift from the early to late phase of Ad replication. This situation could prolong transcription of early Ad genes, further promoting AAV growth which is dependent only on early Ad genes (13, 26, 27).

A possible mechanism for AAV suppression of Ad replication might involve competition for binding of the Ad DBP. Binding of DBP to AAV DNA (or even to AAV RNA) could restrict its availability for Ad DNA synthesis, which is associated with the coating of displaced single strands by the DBP (36). Our data imply that AAV protein does not play a significant role in the suppression of Ad DNA synthesis, since the DBP immunofluorescent staining pattern that correlates with restricted Ad DNA synthesis also occurs in CV-C cells coinfected only with AAV (i.e., without additional SV40 infection), a condition in which AAV protein synthesis is highly inhibited.

Kruijer et al. (20) recently reported the base sequence of the Ad5WT DBP gene. They also sequenced the DBP genes of ts125 and hr404 (an Ad5 mutant which grows autonomously in normally restrictive monkey cells) and found that the DBP genes of these mutants contain different single base changes. For ts125 the mutation maps near the carboxy terminus of the DBP molecule in the large or 46,000-dalton DNA-binding fragment (17). On the other hand, the hr404 mutation maps close to the amino terminus in a domain devoid of cysteines but rich in proline and charged amino acids. This latter domain is contained within the remaining small or 26,000-dalton fragment that does not bind to DNA (17). A vital role for the large DBP fragment in Ad DNA synthesis is clear both in vivo (since ts125 is DNA-minus) and in vitro (12, 16). The small fragment, however, serves a different function, because the monkey cell restriction for Ad and AAV replication is corrected by a mutation that maps in this fragment, (hr404), although both AdWT and AAV synthesize their DNA abundantly in monkey cells without enhancement by SV40 (4).

Early work ascribed the Ad restriction in monkey cells to a reduction in the translation of several late Ad mRNAs in vivo, because translatable Ad mRNA was present in CV-1 cells (9, 10). Klessig and Chow (18) recently proposed that the block to Ad expression in monkey cells is at the level of mRNA processing. They found abnormalities of Ad mRNA splicing in CV-1 cells which suggested that some mRNA species are correctly processed whereas others (particularly fiber mRNA) are not unless the cells are coinfected with SV40. Zorn and Anderson (38) used cell fusion to demonstrate that the block to Ad growth in monkey cells is apparently due to the lack of a promoter-like activity supplied by permissive human cells. Thus, it appears that the synthesis of several late Ad proteins in monkey cells depends upon a site in the small DBP fragment which may interact with some host factor to ensure that certain Ad mRNAs are properly spliced and, perhaps, effectively translated.

Our results suggest that the same DBP site also may be required for translation of AAV mRNA, since AAV protein is deficient in CV-C cells that contain abundant amounts of translatable AAV mRNA (Fig. 3). The mechanism of this DBP activity might involve direct interaction with AAV mRNA. Binding of Ad DBP to mRNA has recently been proposed by Babich and Nevins (2), who observed that the abundance and turnover of early Ad mRNA is dependent upon a functional DBP. We suggest the possibility that in nonpermissive monkey cells normal human Ad DBP binds through its large component to AAV mRNA, but because a specific interaction with a host factor does not occur, efficient translation does not proceed. However, a functional or structural modification of a site within the small component as a result of SV40 coinfection or by mutation, as in hr404, is corrective. Furthermore, since recent DNA transfection experiments have indicated that efficient AAV mRNA translation requires both Ad DBP and Ad VA I RNA (J. E. Janik, M. M. Huston, K. Cho, and J. A. Rose, Abstr. 11th Annu. UCLA Symp. 1982, no. 0580, p. 209), it seems reasonable to consider that VA I RNA also plays an important role in the translation of several late Ad proteins.

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