Independent, Spontaneous Mutants of Adenovirus Type 2-Simian Virus 40 Hybrid Ad2⁺ND3 That Grow Efficiently in Monkey Cells Possess Identical Mutations in the Adenovirus Type 2 DNA-Binding Protein Gene

CARL W. ANDERSON,^{1*} MEDORA M. HARDY,¹ JOHN J. DUNN,¹ AND DANIEL F. KLESSIG²

Department of Biology, Brookhaven National Laboratory, Upton, New York 11973,¹ and Department of Cellular, Viral, and Molecular Biology, University of Utah, Salt Lake City, Utah 84132²

Received 15 April 1983/Accepted 30 June 1983

Four independent, spontaneous mutants of the adenovirus type 2-simian virus 40 hybrid Ad2⁺ND3 that allow efficient growth in monkey cells were isolated previously (C. W. Anderson, *Virology* 111:263-269, 1981). All four mutations have been mapped within the coding sequence for the adenovirus DNA-binding protein by marker rescue analysis. DNA sequence analysis of a region of ca. 1,000 base pairs shown by marker rescue to contain the host range mutations demonstrated that the host range mutant *hr602* differs from its parent, Ad2⁺ND3, at only a single nucleotide. Mutant *hr602* has a thymine in place of a cytosine at the first position of the 130th codon, as measured from the initiation site for the DNA-binding protein. This change results in the replacement of a histidine by a tyrosine in mutant *hr602* DNA-binding protein. Each of the other three Ad2⁺ND3 host range mutants have exactly the same nucleotide alteration as does *hr602*. This same nucleotide change was recently reported for a similarly derived host range mutant of adenovirus 5 (W. Kruijer et al., *Nucleic Acids Res.* 9:4439-4457, 1981).

Although human adenoviruses grow poorly in cell cultures derived from African green monkey kidneys or Rhesus monkey kidneys (25,29), viral variants may be obtained that yield 100- to 1,000-fold more infectious virus per infected cell than is obtained with the parent wild-type virus (1, 13, 20). The first such variants to be isolated were hybrid viruses formed between adenovirus and simian virus 40 (SV40) (20, 21; see reference 29 for a review). These variants had various portions of the SV40 genome covalently integrated into the adenovirus genome and a concomitant deletion of some adenovirus DNA. One series of hybrid viruses isolated by Lewis and his colleagues (20, 21) maintained its ability to grow in human cells without helper virus; these were termed nondefective (ND) hybrids. Those that were also able to grow efficiently in monkey cells were found to express the carboxy-terminal portion of the SV40 large tumor antigen (9). More recently, a second type of adenovirus host range variant was isolated by serial passage of adenovirus stocks in SV40-free monkey cells (1, 13). The mutation(s) carried by some of these variants have been mapped by marker rescue analysis to the coding sequence for the adenovirus 72,000-molecular-weight DNA-binding protein (DBP) (15). The nucleo-

tide sequence of one such mutant, Ad5*hr404*, was recently reported (18).

We recently reported the isolation and partial characterization of four independent, spontaneous mutants of the hybrid virus Ad2⁺ND3 that grow efficiently in cultured monkey cells (1). Ad2⁺ND3 is an adenovirus 2 (Ad2)-SV40 hybrid that does not contain coding information for the SV40 T antigen and therefore grows as poorly in monkey cells as does wild-type Ad2. To further characterize these mutants and the mechanisms by which adenovirus may overcome the block to efficient replication in monkey cells, we characterized these four mutants by both marker rescue and DNA sequence analysis.

MATERIALS AND METHODS

Cells and virus. The origins of our adenovirus stocks, the isolation of the four Ad2⁺ND3 host range mutants, *hr600*, *hr601*, *hr602*, and *hr603*, and the propagation of human HeLa and African green monkey kidney cell lines CV-1 and CV-C have been described previously (1). Briefly, the four mutants were isolated from a recently plaque-purified stock of Ad2⁺ND3 by serial passage in CV-1 cells, followed by plaque isolation on CV-1 and HeLa cells and further propagation in HeLa cells. Each mutant was derived from a separate serial passage experiment. Viral DNA was isolated from cesium chloride gradient-purified

virus as described by Pettersson and Sambrook (24).

Marker rescue. Marker rescue analysis was performed by the calcium phosphate-DNA precipitate transfection method of Graham and van der Eb (7) and has been described in detail previously (15). Restriction fragments produced from genomic DNA were separated by agarose gel electrophoresis. After electroelution and phenol extraction, individual fragments were mixed with adenovirus type 5 (Ad5) DNA and precipitated, and the precipitates were used to transfect the human 293 cell line. Virus obtained from these transfections was propagated by three cycles of infection of CV-1 cells, and the third CV-1 stock was assayed for infectious virus by plaque assay on HeLa and CV-1 cells. Each marker rescue experiment was performed in duplicate or triplicate, and appropriate positive and negative controls were included.

DNA sequence analysis. DNA sequences were determined by the method of Maxam and Gilbert (22). DNA restriction fragments, derived from ca. 100 to 200 μ g of purified viral DNA, were dephosphorylated and then labeled by incubation with bacteriophage T4 kinase and [γ - 32 P]ATP. After cleavage with an appropriate restriction enzyme, the relevant fragments were fractionated by agarose or acrylamide gel electrophoresis. Individual isolated fragments were divided into five parts and treated to allow the determination of adenosine (A) and cytosine (C), guanosine (G), A plus G, C plus thymidine (T), and C residues by gel electrophoresis of the treated samples. Each treated sample was analyzed by electrophoresis through gels of four different acrylamide-bisacrylamide concentrations; after electrophoresis, each gel was subjected to autoradiography at -70°C with Cronex 2 film (Dupont Co.) and Quanta III intensifying screens (Dupont Co.). In general, 200 to 250 nucleotides of contiguous sequence were determined for each labeled fragment. The general strategy of our sequence analysis is outlined in Fig. 3. The nucleotide sequence of the wild-type Ad2 DBP was recently reported by Kruijer et al. (19).

RESULTS

Marker rescue analysis. To determine which region of the genome of each mutant was responsible for the extended host range phenotype, we digested DNA from each mutant virus with restriction enzymes, separated individual fragments by gel electrophoresis, and mixed these purified fragments with Ad5 DNA and used them to transfect human 293 cells. Human 293 cells were chosen for transfection because previous experience indicated that transfection by adenovirus DNA was much more efficient with these cells than with CV-1 cells (15). To select recombinants, we propagated viruses obtained from transfections by three cycles of infection of CV-1 monolayer cultures. CV-1 monkey cells yield 100- to 1,000-fewer infectious adenovirus particles per cell than do HeLa cells. The third passage of each transfection was assayed for infectious virus on both CV-1 and HeLa cell monolayers. Under these conditions, transfections with Ad5 DNA alone gave 10^6 to 10^7 PFU/ml of lysate when assayed on HeLa cell mono-

layers and ca. 10^4 PFU/ml of lysate when assayed on CV-1 cell monolayers.

It should be noted that the spontaneous frequency with which Ad5 host range variants are generated is sufficiently large so that occasionally somewhat larger yields of adenovirus were obtained. Consequently, each transfection experiment was done in duplicate or triplicate. The spontaneous frequency with which Ad2 produces host range variants is apparently much lower than that of Ad5. The reason for this apparent difference between these two very closely related serotypes is unknown.

The patterns of fragments obtained after cleavage of Ad2 or Ad2⁺ND3 DNA with the restriction enzymes used to generate fragments for marker rescue analysis are shown in Fig. 1. The Ad2⁺ND3 restriction maps for these enzymes are given in Fig. 2. The ability of each *Eco*RI restriction fragment generated from the four Ad2⁺ND3 host range mutants to produce recombinants with Ad5 that allow efficient growth in monkey cells is given in Table 1. It is obvious that for each mutant only the *Eco*RI B fragment, spanning the region from 58.5 to 70.7 map units (mu), gave significant numbers of recombinants that grew better in monkey cells than did Ad5. This result was confirmed for mutants *hr600* and *hr601* by analysis of the *Bam*HI restriction fragments (Table 2). Only *Bam*HI-A1 (59.0 to 80.6 mu plus 156 nucleotides of the SV40 sequence) produced Ad5 recombinants with enhanced growth potential in monkey cells. Fragments A1 and A2 result from *Bam*HI cleavage within the SV40 insertion in Ad2⁺ND3 that replaces part of the E3 region of Ad2 (80.6 to 85.5 mu) (11; S. Zain, personal communication).

To further refine the map location of these host range mutants, marker rescue analysis was performed with restriction fragments generated by combined cleavage with the restriction enzymes *Eco*RI and *Bgl*II (Table 3) and by cleavage with the enzyme *Xho*I (Table 4). Only the mixture of *Eco*RI-*Bgl*II fragments containing sequences from 63.6 to 70.7 mu (fragment C*) and from 77.9 to 89.9 mu (fragment F-I) gave efficient marker rescue. Because the host range mutations must reside in the region shared with both *Eco*RI B (58.5 to 70.7 mu) and *Bam*HI A1 (59.5 to 80.3 mu) fragments, the C* fragment rather than the F-I fragment is responsible for rescue of virus growth. Thus, the host range mutation in each mutant must be within the sequence from mu 63.6 to 70.7. This region of the adenovirus genome encodes the amino-terminal portion of the 72,000-*M_r* DBP and the amino-terminal portion of the 100,000-dalton late, nonvirion protein.

Marker rescue experiments with *Xho*I-derived

FIG. 1. Ad2⁺ND3 restriction fragments used for marker rescue. Photographs of ethidium bromide-stained agarose gels after electrophoresis of Ad2 or Ad2⁺ND3 DNA digested with the indicated restriction enzymes are shown. The bands are labeled as described in the legend to Fig. 2. Fusion bands are designated according to the fragments forming their left and right ends. Two small fragments from the *Eco*RI-*Bgl*II double digest have been arbitrarily designated fragments 10 and 11. Fragment C* is the left portion of the *Bgl*II C fragment which is cut by *Eco*RI at 70.7 mu.

fragments from mutants *hr600*, *hr602*, and *hr603* indicated that the mutations in these mutants must lie to the left of map position 66.0 (Table 4). Recent sequence analysis of the region encoding the DBP of Ad2 (19) indicates that the *Xho*I site at 66.0 is situated 50 codons downstream from the AUG triplet believed to be used for the initiation of DBP synthesis. Thus, the marker rescue analyses indicated that the host range mutations in *hr600*, *hr602*, and *hr603* must lie within the coding region for the adenovirus DBP.

Two attempts at marker rescue with *Xho*I-derived fragments from mutant *hr601* failed. The reason for these failures probably reflects technical problems rather than any significant difference in the *hr601* genome or the mutation carried by it. We noted considerable difficulty in obtaining complete digestion at the 66.0-mu site with *Xho*I. The reason for this difficulty is unknown. Incomplete cleavage at the 66.0-mu site produced a partial digestion product that was not

resolved from the mixture of the two large *Xho*I fragments (A and D-B) that flank the *Xho*I E fragment (53.0 to 66.0 mu). Indeed, rescued virus was obtained with this fragment mixture in several *Xho*I marker rescue experiments. A second factor that may have contributed to our failure to obtain many rescued recombinants with the *Xho*I E fragment from *hr601* DNA is that the site at 66.0 mu is close to the host range mutation carried by all mutants (see below). Since the probability of recombination between the mutation and the right end of the *Xho*I E fragment is low, only experiments in which transfection and recombination are efficient would be expected to yield unambiguous results.

Nucleotide sequence analysis of the Ad2⁺ND3 host range mutants. To determine the precise location and nature of the mutation carried by each host range mutant, DNA sequence analysis was performed. The strategy for determining the complete nucleotide sequence between the *Bgl*II site at 63.6 mu and the *Xho*I site at 66.0 mu for

FIG. 2. Restriction map of the Ad2⁺ND3 genome. The double lines labeled AD2 and ND3 near the top of the figure represent the genomes of AD2 and Ad2⁺ND3, respectively. Adenovirus sequences from region E3 between map positions 80.6 and 85.5 are deleted from the Ad2⁺ND3 genome and are replaced by ca. 230 nucleotides of the SV40 sequence (black region) beginning at the termination codon for the SV40 large tumor antigen (S. Zain, personal communication). Arrow-headed lines above the genomic representations show the major adenovirus transcripts, including the major late transcript beginning at 16.45 mu. Below the genomic representations are restriction maps for the enzymes *Eco*RI, *Bam*HI, *Bgl*II, and *Xho*I. Fragments are named according to the standard adenovirus nomenclature (29). Note, however, that the deletion of adenovirus sequences between 80.6 and 85.5 mu results in the loss of the *Eco*RI restriction site at 84.0 mu, the loss of three *Bgl*II restriction sites, and the loss of the *Xho*I site at 83.0 mu. The SV40 insert contains a *Bam*HI site that effects division of the Ad2 *Bam*HI A fragment into two fragments. Thickened, darkened sections indicate regions from the genomes of Ad2⁺ND3 host range mutants that result in recombinants capable of efficient growth in monkey cells. Only the left portion of the *Bgl*II C fragment is so marked, as fragments digested with both *Bgl*II and *Eco*RI were used for marker rescue experiments (see Table 3). Map units given for Ad2⁺ND3 are the same as those given for Ad2 and have not been corrected for the somewhat shorter genome of this hybrid.

wild-type Ad2⁺ND3 and mutant *hr602* DNA is given in Fig. 3. This region of the Ad2⁺ND3 sequence was found to be identical to that recently published by Kruijer et al. (19) for this same region of Ad2 DNA. The complete se-

quence of this region was also determined for the host range mutant *hr602*. Only one nucleotide difference was observed (Fig. 4). Mutant *hr602* has a T residue in place of a C residue as the first base of the 130th codon in the DBP

TABLE 1. Marker rescue by *Eco*RI restriction fragments from Ad2⁺ND3 host range mutants

Ad2 ⁺ ND3 host range mutant	Marker rescue by the following fragments (map coordinates):				
	A (0-58.5)	B (58.5-70.7)	F (70.7-75.9)	D-E (75.9-89.7)	C (89.7-100)
<i>hr600</i> ^a	0.3	40	1.5	0.3	0.1
<i>hr601</i> ^b	6.6	943	1.0	6.6	6.6
<i>hr602</i> ^a	10.6	403	3.4	3.4	3.4
<i>hr603</i> ^a	0.4	2,098	1.0	44	8

^a Ratio of plaques obtained on CV-1 monolayers from the third CV-1 passage of transfection of 293 cells with the indicated fragment plus Ad5 DNA to plaques obtained from the third passage of transfection with Ad5 DNA alone (3.5×10^2 PFU/ml for *hr600*, 1.6×10^4 PFU/ml for *hr602*, and 3×10^3 PFU/ml for *hr603*). Numbers represent the average of two or three independent transfections for each fragment.

^b Ratio of plaques obtained on CV-1 monolayers from the third CV-1 passage of transfection of 293 cells with Ad5 DNA plus the indicated fragment to plaques obtained from the third passage of transfection with Ad5 DNA and the *Eco*RI F fragment (3×10^3 PFU/ml for *hr601*). Numbers represent the average of two or three independent transfections for each fragment.

TABLE 2. Marker rescue by *Bam*HI restriction fragments from Ad2⁺ND3 host range mutants

Ad2 ⁺ ND3 host range mutant	Marker rescue by the following fragments (map coordinates):				
	B (0-29.1)	D (29.1-40.9)	C (40.9-59.0)	A1 ^a (59.0-80.6)	A2 ^a (85.5-100)
<i>hr600</i> ^b	<0.2	6	<0.2	57	<0.2
<i>hr601</i> ^c	6	1	1	22	6

^a The *Bam*HI site that separates fragments A1 and A2 is in the 156-bp SV40 sequence that replaces the adenovirus sequence between 80.6 mu and 85.5 mu in the Ad2⁺ND3 hybrid virus genome.

^b Ratio of plaques obtained on CV-1 monolayers from the third CV-1 passage of transfection of 293 cells with Ad5 DNA plus the indicated fragment to plaques obtained from the third passage of transfection with Ad5 DNA alone (2.1×10^2 PFU/ml). Numbers represent the average of three independent transfections for each fragment.

^c Ratio of plaques obtained on CV-1 monolayers from the third CV-1 passage of transfection of 293 cells with Ad5 DNA plus the indicated fragment to plaques obtained from the third passage of transfection with Ad5 DNA plus the *Bam*HI C fragment (8.8×10^3 PFU/ml). Numbers represent the average of three independent transfections for each fragment.

gene, which changes the triplet from a histidine codon to a tyrosine codon. No other difference between the nucleotide sequences of the wild-type and mutant DBPs has been observed.

To analyze the nucleotide sequence of the other three host range mutants, we first examined the sequence on either side of the *Hpa*II site at 65.5 mu. All three mutants were found to have precisely the same nucleotide alteration as did *hr602*. No other difference was found in the sequences of these mutants within the region examined, about 200 base pairs (bp) on either side of this *Hpa*II site. Thus, all four host range mutants of Ad2⁺ND3, *hr600*, *hr601*, *hr602*, and *hr603*, carry precisely the same mutation.

DISCUSSION

We sequenced a contiguous 1,085-nucleotide segment of the genome of the hybrid virus Ad2⁺ND3 that corresponds to the amino-terminal two-thirds of its DBP. This sequence is identical to the equivalent region of the Ad2 sequence recently published by Kruijer et al. (19). Although 638 nucleotides corresponding to the carboxy-terminal segment of the DBP gene of Ad2⁺ND3 have not been determined (see legend to Fig. 3), it seems likely that the entire

amino acid sequences of Ad2⁺ND3 and Ad2 DBP are identical since all of the differences between the amino acid sequences of Ad2 DBP and the DBP of the closely related Ad5 lie in the sequenced amino-terminal region. The Ad2 sequences in Ad2⁺ND3 originated from an adenovirus isolate designated strain Ad.6 (adenoid no. 6) (20, 21). The virus studied by Kruijer et al. (19) may also be derived from this isolate (L. Philipson, personal communication).

Only one nucleotide difference between wild-type Ad2⁺ND3 and the host range mutant *hr602* was observed in the sequenced region between the *Bgl*II site at 63.6 mu and the *Xho*I site at 66.0 mu. This segment of the DBP gene contains the mutation responsible for the increased growth efficiency of *hr602* in monkey cells, since the transfer of this segment to Ad5 by marker rescue transferred the growth characteristics of *hr602* to Ad5 (see Fig. 2). The substitution of a T residue for a C residue as the first nucleotide of the 130th codon of the DBP gene resulted in the replacement of a histidine in the wild-type DBP with a tyrosine in the mutant.

Sequence analysis of three additional mutants of Ad2⁺ND3 revealed that each has precisely the same nucleotide alteration as does *hr602*. No

TABLE 3. Marker rescue by *Bgl*II-*Eco*RI restriction fragments^a from Ad2⁺ND3 host range mutants

Ad2 ⁺ ND3 host range mutant ^b	Marker rescue by the following fragments (map coordinates):					
	Bgl-D* (45.3-58.5)	10 (58.5-60.2)	Bgl-J (60.2-63.6)	Bgl-C*-F-I ^c (63.6-70.7, 77.9-89.9)	RI-F-Bgl-G ^c (70.7-75.9, 89.9-95.0)	All (digest mix)
<i>hr600</i>	14	2.1	1.9	3,600	32	8,500
<i>hr601</i>	2.3	2.0	2.5	3,750	15	500
<i>hr602</i>			0.1	1,320	0.8	680
<i>hr603</i>			0.5	2,600	0.4	630

^a Fragments resulted from simultaneous digestion by restriction enzymes *Eco*RI and *Bgl*II.

^b Ratio of plaques obtained on CV-1 monolayers from the third CV-1 passage of transfection of 293 cells with Ad5 DNA plus the indicated fragment(s) to plaques obtained from the third passage of transfection with Ad5 DNA alone. Numbers represent the average of two or three independent transfections for each fragment.

^c Unresolved fragment mixtures (see Fig. 1).

TABLE 4. Marker rescue by *Xho*I restriction fragments from Ad2⁺ND3 host range mutants

Ad2 ⁺ ND3 host range mutant ^a	Marker rescue by the following fragments (map coordinates):			
	G (22.0–26.5)	A–D–B ^b (26.5–53.0, 66.0–100)	E (53.0–66.0)	All (digest mix)
<i>hr600</i>		1.3	2,620	37
<i>hr602</i>	1.0	3.0	300	36
<i>hr603</i>	1.1	34	460	3,000

^a Ratio of plaques obtained on CV-1 monolayers from the third CV-1 passage of transfection (of 293 cells) with Ad5 DNA plus the indicated fragment(s) to plaques obtained from the third passage of transfection with Ad5 DNA alone. Numbers represent the average of two independent transfections for each fragment.

^b Fragment mixture; fragments D and B run as a single fragment owing to the deletion of the Ad2 *Xho*I site at 83.0 mu from the Ad2⁺ND3 genome. This fragment was not resolved from the *Xho*I A fragment under the electrophoresis conditions used (see Fig. 1).

other changes were observed in the genomes of *hr600*, *hr601*, and *hr603* within 160 bp on either side of the altered codon 130. Although it is impossible to rigorously prove that the four mutants are independent, the fact that they were isolated from stocks passaged independently in monkey cells strongly suggests that this is the

case (1). The finding by Kruijer et al. (18) that *hr404*, a host range mutant of Ad5, has exactly the same nucleotide alteration as does *hr602* indicates that at least two independent host range mutants, and most probably five mutants, have precisely the same nucleotide alteration.

Histidine codon 130 (CAC in Ad2 and CAT in

FIG. 3. Sequencing strategy for identifying the host range mutation in Ad2⁺ND3 *hr602*. The region of the Ad2 genome from the *Kpn*I site at 61.3 mu (N631) extending 2,000 bp towards the right is depicted; this region encodes the adenovirus DBP. Nucleotide positions given in this text are according to the numbering system of Kruijer et al. (19). Marker rescue experiments place the Ad2⁺ND3 host range mutations between the *Bgl*II site at 63.6 mu and the *Xho*I site at 66.0 mu (vertical arrows). The thickened line indicates the region of the Ad2⁺ND3 genome that was sequenced. A contiguous 1,085-bp sequence between N1262 and N2346 was obtained. An additional 173 nucleotides of sequence between the *Bgl*II site at 63.6 mu and N1086 were obtained. These 1,258 nucleotides, extending from N1089 to N2346, were found to be identical to the sequence published by Kruijer et al. (19) for wild-type Ad2. The comparable region of Ad2⁺ND3 *hr602* was identical to the sequence of the wild-type hybrid except for a single nucleotide change at N1913 (19) (see text and Fig. 4). Whole genomic DNA was digested with *Bgl*II, *Bst*EII, or *Xho*I, labeled with [³²P]ATP using polynucleotide kinase, and then subcut with *Kpn*I, *Hpa*II, or *Eco*RI, respectively. The appropriate fragments, now labeled at only one end, were isolated after agarose or polyacrylamide gel electrophoresis. The remaining fragments were obtained by first preparing the *Eco*RI B or *Kpn*I E fragments from genomic DNA. Labeled *Sst*II fragments were subcut with *Taq*I, labeled *Hpa*II fragments were subcut with *Bst*EII, and labeled *Taq*I fragments were subcut with *Dde*I. Vertical lines represent restriction sites; arrows indicate the regions and the strand for which sequence information was obtained.

FIG. 4. Sequence analysis of the host range mutation in Ad2⁺ND3 *hr602*. A portion of the sequencing gel autoradiogram that displays the single nucleotide change in the DBP gene of host range mutant *hr602* is shown. The *Kpn*I E fragment from wild-type Ad2⁺ND3 or *hr602* DNA was digested with *Hpa*II, labeled using polynucleotide kinase, and subcut with *Bst*EII. The isolated fragments, labeled at the *Hpa*II site at N1925 and extending to N1530 (19), were subjected to the five sequencing reactions described by Maxam and Gilbert (22). Nucleotide 1,913, the first nucleotide of a histidine codon at position 130 from the initiation site for DBP synthesis, is clearly shown to be altered from a C to a T in *hr602*. This alteration causes the replacement of this histidine by a tyrosine in the mutant DBP. A similar experiment with the other three host range mutants (*hr600*, *hr601*, and *hr603*) indicated that each of these mutants was altered in exactly the same way.

Ad5) can be changed in a single step to encode seven different amino acids (tyrosine, asparagine, aspartic acid, leucine, proline, arginine, and glutamine). That only tyrosine has been observed in the five mutants that have been sequenced presumably indicates that tyrosine at this position is important to the host range function. We have not observed any difference in the phosphorylation pattern of wild-type or mutant DBP isolated from monkey or human cells (data not shown). This failure to detect a difference is not surprising since the presence of a large number of other phosphates on this heavily phosphorylated protein (12, 27) could easily mask any changes in post-translational modifications that occurred at amino acid 130 or because of the alteration of amino acid 130. Efforts to identify the Tyr130-containing peptide are in progress.

Structural studies of the adenovirus DBP provide evidence for two physically distinct domains. The DBP is an elongated protein having an axial ratio of >10:1 (28, 30). Digestion of this protein with a variety of proteolytic enzymes

produces similar carboxy-terminal segments that retain several biological activities (6, 12, 28). These functions include the capacity to bind to single-stranded DNA and the ability to support the elongation reaction of DNA replication in vitro. Most of the phosphorylation sites within the DBP are located in an amino-terminal segment. The position of the new tyrosine in the host range mutants is within the amino-terminal region of the protein but lies close to the chymotrypsin site that demarks the functional carboxy-terminal segment (C. W. Anderson and A. J. Levine, unpublished data). It also lies at the beginning of a 38-amino acid-long hydrophilic segment that could act as a hinge between the two physical domains.

Genetic analysis of mutations within the DBP gene supports the concept of two functionally independent domains. Temperature-sensitive mutations and their revertants in the DBP gene define a carboxy-terminal region required for DNA binding, DNA replication, and the turning off of early gene expression (3, 4, 16–19, 23, 31; S. Rice and D. F. Klessig, submitted for publica-

tion). In contrast, host range mutations which allow efficient growth in monkey cells reside in the segment of the DBP gene coding for the amino-terminal region. These two regions appear to be functionally independent since a DBP that is both temperature sensitive for DNA binding and carries the host range alteration permits efficient viral growth in monkey cells at an elevated temperature (16; Rice and Klessig, submitted for publication).

Adenovirus-infected monkey cells express reduced levels of most late proteins; in particular, fiber protein synthesis is severely depressed (5, 8, 9, 14). Much of the reduction in late gene expression can be accounted for by a reduction in late mRNA levels, but at least in the case of fiber protein, there also appears to be a block at the level of mRNA translation. Fiber mRNA is not translated in abortively infected monkey cells even though it is associated with polysome-like structures; however, this RNA can be translated *in vitro* (2). DBP function is also necessary for the expression of adeno-associated virus late proteins (10, 26). Whether the role(s) DBP plays in controlling adeno-associated virus late protein synthesis or adenovirus early gene expression in human cells is related to its function in overcoming the block to late gene expression and virus reproduction in monkey cells remains to be determined.

ACKNOWLEDGMENTS

We acknowledge the expert technical assistance of S. Lamb, A. Lewis, W. Crockett, and V. Cleghon.

This work was supported by Public Health Service grant 5R01AI17315 from the National Institutes of Health to D.F.K., Program Project grant CA28146 from the National Institutes of Health, and by the U.S. Department of Energy.

ADDENDUM IN PROOF

Sequence analyses by D. Brough and D. F. Klessig indicate that the same histidine-to-tyrosine alterations found in *hr600*, *hr601*, *hr602*, and *hr603* also occur in the independently derived host range mutants Ad2 *hr400* through *hr403* (15).

LITERATURE CITED

- Anderson, C. W. 1981. Spontaneous mutants of the adenovirus-simian virus 40 hybrid, Ad2*ND3, that grow efficiently in monkey cells. *Virology* 111:263-269.
- Anderson, K. P., and D. F. Klessig. 1983. Post-transcriptional block to synthesis of a human adenovirus capsid protein in abortively infected monkey cells. *J. Mol. Appl. Genet.* 2:31-43.
- Babich, A., and J. R. Nevins. 1981. The stability of early adenovirus mRNA is controlled by the viral 72kd DNA binding protein. *Cell* 26:371-379.
- Blanton, R. A., and T. H. Carter. 1979. Autoregulation of adenovirus type 5 early gene expression. III. Transcription studies in isolated nuclei. *J. Virol.* 29:458-465.
- Farber, M. S., and S. G. Baum. 1978. Transcription of adenovirus RNA in permissive and nonpermissive infections. *J. Virol.* 27:136-148.
- Friefeld, B. R., M. D. Krevolin, and M. S. Horwitz. 1983. Effects of the adenovirus H5ts125 and H5ts107 DNA binding protein on DNA replication *in vitro*. *Virology* 124:380-389.
- Graham, F. L., and J. A. van der Eb. 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 52:456-467.
- Grodzicker, T., C. Anderson, P. A. Sharp, and J. Sambrook. 1974. Conditional lethal mutants of adenovirus 2-simian virus 40 hybrids. I. Host range mutants of Ad2*ND1. *J. Virol.* 13:1237-1244.
- Grodzicker, T., J. B. Lewis, and C. W. Anderson. 1976. Conditional lethal mutants of adenovirus type 2-simian virus 40 hybrids. II. Ad2*ND1 host-range mutants that synthesize fragments of the Ad2*ND1 30K protein. *J. Virol.* 19:559-571.
- Jay, F. T., C. A. Laughlin, and B. J. Carter. 1981. Eukaryotic translational control: adeno-associated virus protein synthesis is affected by a mutation in the adenovirus DNA-binding protein. *Proc. Natl. Acad. Sci. U.S.A.* 78:2927-2931.
- Kelly, T. J., Jr., and A. M. Lewis, Jr. 1973. Use of nondefective adenovirus-simian virus 40 hybrids for mapping the simian virus 40 genome. *J. Virol.* 12:643-652.
- Klein, H., W. Maltzman, and A. J. Levine. 1979. Structure-function relationships of the adenovirus DNA-binding protein. *J. Biol. Chem.* 254:11051-11060.
- Klessig, D. F. 1977. Isolation of a variant of human adenovirus serotype 2 that multiplies efficiently on monkey cells. *J. Virol.* 21:1243-1246.
- Klessig, D. F., and C. W. Anderson. 1975. Block to multiplication of adenovirus serotype 2 in monkey cells. *J. Virol.* 16:1650-1668.
- Klessig, D. F., and T. Grodzicker. 1979. Mutations that allow human Ad2 and Ad5 to express late genes in monkey cells map in the viral gene encoding the 72K DNA binding protein. *Cell* 17:957-966.
- Klessig, D. F., and M. P. Quinlan. 1982. Genetic evidence for separate functional domains on the adenovirus specified, 72 kd DNA-binding protein. *J. Mol. Appl. Genet.* 1:263-272.
- Kruijjer, W., J. C. Nicolas, F. M. A. van Schaik, and J. S. Sussenbach. 1983. Structure and function of DNA binding proteins from revertants of adenovirus type 5 mutants with a temperature-sensitive DNA replication. *Virology* 124:425-433.
- Kruijjer, W., F. M. A. van Schaik, and J. S. Sussenbach. 1981. Structure and organization of the gene coding for the DNA binding protein of adenovirus type 5. *Nucleic Acids Res.* 9:4439-4457.
- Kruijjer, W., F. M. A. van Schaik, and J. S. Sussenbach. 1982. Nucleotide sequence of the gene encoding adenovirus type 2 DNA binding protein. *Nucleic Acids Res.* 10:4493-4500.
- Lewis, A. M., Jr., M. J. Levin, W. H. Wiese, C. S. Crumpacker, and P. H. Henry. 1969. A non-defective (competent) adenovirus-SV40 hybrid isolated from the Ad2-SV40 hybrid population. *Proc. Natl. Acad. Sci. U.S.A.* 63:1128-1135.
- Lewis, A. M., Jr., A. S. Levine, C. S. Crumpacker, M. J. Levin, R. J. Samaha, and P. H. Henry. 1973. Studies of nondefective adenovirus 2-simian virus 40 hybrid viruses. V. Isolation of additional hybrids which differ in their simian virus 40-specific biological properties. *J. Virol.* 11:655-664.
- Maxam, A. M., and W. Gilbert. 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol.* 65:499-560.
- Nevins, J. R., and J. J. Winkler. 1980. Regulation of early adenovirus transcription: a protein product of early region 2 specifically represses region 4 transcription. *Proc. Natl. Acad. Sci. U.S.A.* 77:1893-1897.
- Pettersson, U., and J. Sambrook. 1973. The amount of viral DNA in the genome of cells transformed by adenovirus type 2. *J. Mol. Biol.* 73:125-130.

25. **Rabson, A. S., G. T. O'Coner, I. K. Berezsky, and F. J. Paul.** 1964. Enhancement of adenovirus growth in African green monkey kidney cell cultures by SV40. *Proc. Soc. Exp. Biol. Med.* **116**:187-190.
26. **Richardson, W. D., and H. Westphal.** 1981. A cascade of adenovirus early functions is required for expression of adeno-associated virus. *Cell* **27**:133-141.
27. **Russell, W. C., and G. E. Blair.** 1977. Polypeptide phosphorylation in adenovirus-infected cells. *J. Gen. Virol.* **34**:19-35.
28. **Schechter, N. M., W. Davies, and C. W. Anderson.** 1980. Adenovirus coded deoxyribonucleic acid binding protein. Isolation, physical properties, and effects of proteolytic digestion. *Biochemistry* **19**:2802-2810.
29. **Tooze, J.** 1980. Molecular biology of tumor viruses, part 2. DNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **van der Vleit, P. C., W. Keegstra, and H. S. Janz.** 1978. Complex formation between the adenovirus type 5 DNA-binding protein and single-stranded DNA. *Eur. J. Biochem.* **86**:389-398.
31. **van der Vleit, P. C., A. J. Levine, M. J. Ensinger, and H. S. Ginsberg.** 1975. Thermolabile DNA binding protein from cells infected with a temperature-sensitive mutant of adenovirus defective in viral DNA synthesis. *J. Virol.* **15**:348-354.