JOURNAL OF VIROLOGY, Feb. 1981, p. 620-627
0022-538X/81/020620-08$02.00/0

Vol. 37, No. 2

Subgenomic Viral DNA Species Synthesized in Simian Cells by Human and Simian Adenoviruses

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DNA synthesized after infection of simian tissue culture cells (BSC-1 or CV-1) with human adenovirus type 2 or 5 or with simian adenovirus 7 was characterized. It was demonstrated that as much as 40% of the virus-specific DNA in nuclei of infected monkey cells consists of subgenomic pieces. No subgenomic viral DNA species were detected in the nuclei of human (HeLa) cells infected with these adenovirus types. Restriction analysis showed that these short viral DNA molecules contain normal amounts of the sequences from the ends of the viral genome, whereas internal regions are underrepresented. The production of subgenomic DNAs is not correlated with semipermissive infection. Although adenovirus types 2 and 5 are restricted in monkey cells, these cells are fully permissive for simian adenovirus 7. HR404, an adenovirus type 5 mutant which is not restricted in monkey cells, produced the same percentage of subgenomic DNAs as did its wild-type (restricted) parent, and coinfection of monkey cells with adenovirus type 5 and simian virus 40 did not reduce the production of subgenomic adenoviral DNAs. The array of predominant size classes among the heterogeneously sized short DNAs is serotype specific. Extensive plaque purification and comparison of wild-type adenovirus type 5 with several viral mutants indicated that the distribution of aberrant sizes of DNA is characteristic of the virus and not a result of random replicative errors and then enrichment of particular species.

Monkey cells are semipermissive for infection by human adenoviruses: events in the viral growth cycle up to and including DNA replication occur with an efficiency comparable to that exhibited during growth in human (permissive) cells (12, 13, 15, 33). However, virus production is reduced by at least two orders of magnitude. The block is more severe for some adenovirus serotypes than for others and is dependent on the multiplicity of infection (1). Yields of some late viral proteins are much reduced in monkey cells (9, 12, 13, 15, 24), and a striking deficiency in several species of late mRNA has been noted (21).

Monkey cells are fully permissive for simian adenovirus 7 (SA7), one of a number of simian adenoviruses which have been isolated and characterized. The structure and productive infectious cycle of SA7 seem to be much the same as those of human adenoviruses (3, 10). We have found that infection of HeLa cells with SA7 results in the production of infectious virus particles at a level of about 10% of that amount produced in monkey cells; the efficiency of adsorption of SA7 to the two cell types is the same (S. Zenda, E. Daniell, and L. Stevens, unpublished data).

During permissive infection of human cells by adenoviruses, several classes of incomplete (noninfectious) particles are produced. These contain pieces of viral DNA smaller than the normal genome and can be separated from complete particles on the basis of their lower buoyant density (2, 4, 25, 30, 39). Analysis of DNA extracted from purified incomplete particles of serotypes 2, 3, and 12 shows that the short viral DNAs are heterogeneous in size, and a number of predominant size classes can be detected by gel electrophoresis. Many molecules contain extended inverted terminal repetitions, and sequences from the left-hand end of the complete viral genome are present in excess (4). SA7 also produces incomplete virions in permissive cells (31, 36). The DNA in these virions has not been extensively characterized.

Individual adenovirus serotypes exhibit characteristic yields of incomplete particles, relative to complete virions. The array of size classes of DNA from incomplete particles of a given serotype is also distinctive (4). In a preparation of adenovirus type 2 (Ad2), 5% of the total particles are incomplete, and 0.1% of the viral DNA recovered is in the incomplete fraction. In contrast, Ad3 incomplete particles may contain 2 to 3% of the total viral DNA in a preparation. In Ad5 preparations, only trace amounts of low-buoy-

ant-density particles are found, and there is no evidence that these contain DNA.

We have isolated short viral DNAs from the nuclei of Ad3-infected HeLa cells (5) and found that the same array of sizes is exhibited as that observed among packaged DNA molecules, suggesting that the size specificity is a result of the replicative errors which generate the short DNAs and not a selection on the basis of packaging. These populations of intracellular subgenomic DNAs contain sequences from both ends of the viral genome, suggesting that the selection of left-end molecules is at the level of packaging.

Purification of DNA from nuclei of monkey cells infected with Ad2, Ad5, or SA7 has shown that, in two lines of monkey tissue culture cells, these three types of adenovirus produce a large proportion of subgenomic viral DNAs. This paper characterizes these subgenomic DNAs and the conditions under which they are produced. The fact that these subgenomic DNAs are produced only in monkey cells was used to advantage to show conclusively that the serotype-specific array of defective DNAs is determined by the wild-type genome and not by the presence of seed amounts of specific small DNAs in defective particles of high-titer virus stocks.

MATERIALS AND METHODS

Cells and viruses. CV-1 cells, a subline of AGMK cells (18), and BSC-1 cells were obtained from Edward Penhoet, and HeLa cells were obtained from Cold Spring Harbor Laboratory. Cells were cultured in plastic dishes in Eagle minimum essential medium or in the Dulbecco modification of that medium (6) supplemented with 5% fetal calf serum (GIBCO Laboratories). Ad2 and Ad5 (Cold Spring Harbor Laboratory), and Ad5 HR404 from Daniel Klessig (20, 22) were propagated on monolayers of HeLa cells. H5*ts*125, obtained from Harry Ginsberg, and H5*ts*1, H5*ts*18, and H5*ts*19, obtained from Jim Williams, were propagated on HeLa cells at 32°C. SA7, obtained from the American Type Culture Collection, was propagated on BSC-1 cells.

Plaque titrations and sequential plaque purification. Plaque titrations were performed as described by Williams (41). Rigorous sequential plaque purifications of Ad5 were performed to obtain virus stocks uncontaminated by incomplete DNA. Serial dilutions of a standard high-titer laboratory seed stock of the virus were plated under agar. Plaques from plates containing no more than 10 plaques were picked through the agar with a Pasteur pipette; the picked plaques were diluted into buffer and sonicated, and serial dilutions were made and sonicated. These dilutions were plated, plaques were picked, etc. The process was repeated six times with no intervening passage of virus. Plaques to be inspected for generation of incomplete DNAs in CV-1 cells were sonicated, titrated, and used to infect monolayers of HeLa cells at a multiplicity of infection of 0.05 PFU per cell. The virus was harvested at 36 h after infection (one round of virus growth), and the resulting stocks were used to infect CV-1 cells.

Purification and analysis of DNA from infected cells. Monolayers of CV-1 or HeLa cells were infected with virus, and DNA was extracted from nuclei isolated at various times after infection as described previously (5). ^{32}P-labeled DNA was prepared by replacing the original medium with phosphate-free Eagle minimum essential medium supplemented with 100 μCi of ^{32}P per 60-mm culture dish. [^{3}H]thymidine (10 μCi/ml) was added to the medium at 10 h after infection to obtain tritium-labeled DNA. Preparative separation of labeled small DNA from full-length genomes was performed by centrifugation through 5 to 20% neutral sucrose gradients (0.05 M Tris [pH 7.5], 0.02 M EDTA, 0.1 M NaCl) for 3.75 h at 40,000 rpm in a Beckman SW41 rotor (15°C).

Gel electrophoresis, transfer of DNA to nitrocellulose, and hybridization. Electrophoresis of cellular and viral DNAs on 0.7% agarose gels, staining, photography, and autoradiography were performed as described previously (4). DNA was transferred from gels to nitrocellulose filter sheets by the method of Southern (37) as modified by Ketner and Kelly (19). Specific viral and cellular DNA probes were labeled by nick translation (26) and hybridized to the filters as described previously (4).

Quantitative sizing. Gels containing ^{32}P-labeled DNA were photographed, sliced with reference to appropriate nonradioactive size markers which had been electrophoresed in adjacent slots, and slices were counted by Cerenkov radiation.

Preparations of labeled DNA in which the amount of DNA in various size ranges was to be measured were treated with RNase (Worthington A grade, 50 μg/ml for 2 h at 37°C) before gel electrophoresis. Additionally, the DNA from the viral peak and trailing regions of selected sucrose gradients (see Fig. 4) was pooled, precipitated, and then electrophoresed. Quantitative results from a given labeled preparation were the same with or without this extra purification.

Restriction enzymes and maps. *Hin*dIII was purchased from New England Biolabs. The positions of cleavage sites were described previously by Sussenbach and Kuijk (38).

RESULTS

Size distribution of viral DNA produced in infected cells. Figure 1 shows the electrophoretic mobility through a 0.7% agarose gel of the DNA extracted from nuclei of CV-1 and HeLa cells at 36 h after infection with Ad2 or Ad5. All of the slots containing DNA from infected cells had a distinctive discrete band of DNA comigrating with viral DNA purified from complete particles. The diffuse band migrated more slowly than did viral DNA, and most of the material at the top of the gel was shown by hybridization and by comparison with mock-infected cells to consist of cellular sequences. In the DNA preparations from monkey cells infected with Ad2 or Ad5, there was a variety of shorter pieces of DNA, in addition to the full-

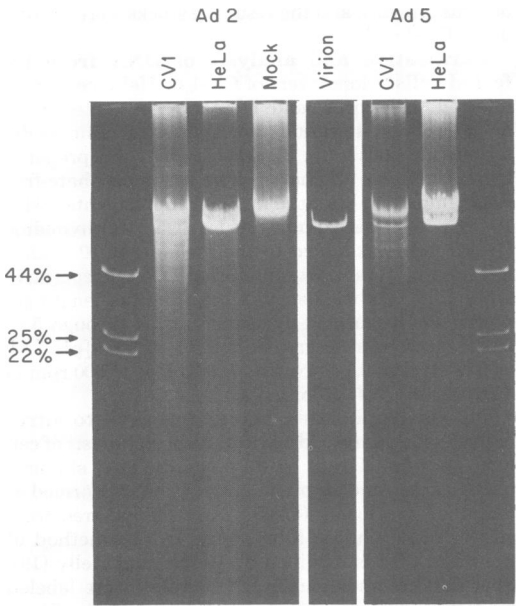

FIG. 1. *Gel electrophoresis of DNA extracted from nuclei of HeLa and CV-1 cells infected with Ad5. DNA was extracted from cells harvested at 30 h after infection and electrophoresed through 0.7% agarose for 15 h at 30 V with 6 μg per slot. Ad5 viral DNA (full length) was extracted from virus particles purified on cesium chloride (1 μg). Fragments from a digest of Ad2 DNA with endonuclease XbaI served as size references. "Mock" DNA is from uninfected CV-1 cells.*

length viral DNAs. These were quite heterogeneous in length, but predominant bands were clearly visible, ranging in size from just under full length to less than 20% of the full-length genome.

To ascertain whether these short DNA fragments consist of cellular or viral sequences, DNAs were transferred from gels to nitrocellulose paper by the method of Southern (37) and specific ^{32}P-labeled viral and cellular DNA probes were hybridized to identical filters. Figure 2a shows the result of such a hybridization in which the probe is nick-translated Ad5 DNA. It is clear that the short DNAs with their heterogeneous array of size classes consist of viral sequences. There was no hybridization of a cellular DNA probe to this region of the filter. It can be concluded then that the specific short DNAs observed in Ad5-infected cells are completely or nearly completely composed of viral sequences.

Quantitation of incomplete viral DNAs produced. To measure the fraction of viral DNAs synthesized which are shorter than full length and to ascertain whether defective DNAs are made preferentially at any particular time during viral DNA replication, infected cells were labeled with ^{32}P at various times after infection. If labeling commences 15 h or more after infection, more than 90% of the label incorporated into DNA is viral, as indicated by liquid hybridization analysis (data not shown).

When labeled DNA from Ad5-infected CV-1 cells was prepared and subjected to electrophoresis as described above, autoradiography of the gel revealed a banding pattern identical to that of the stained, photographed gel and of the blotted, hybridized arrays. For quantitation, radioactive DNA was electrophoresed with appropriate size markers, and the gel was sliced and counted. Table 1 shows the results of four separate labeling experiments of this type. The fraction of total counts which was shorter than full-genome length varied from 0.21 to 0.45, but the variation was not an obvious function of the virus stock used or of the labeling interval. Table 2 shows in more detail the fraction of counts in various size classes.

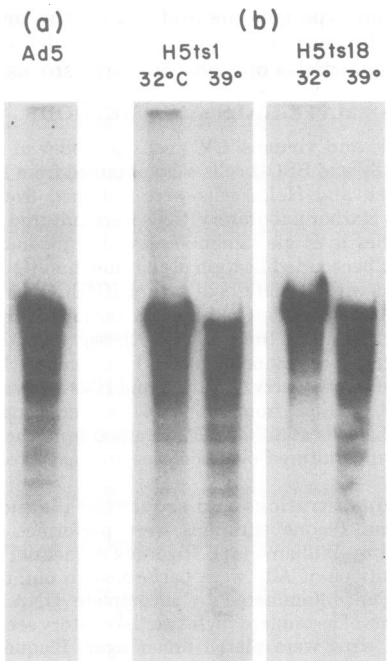

FIG. 2. *Hybridization of ^{32}P-labeled Ad5 DNA to blots of DNA from infected cells. DNA from BSC-1 cells infected with Ad5 was prepared and electrophoresed as in Fig. 1 (a total of 4 μg per slot) and then transferred to nitrocellulose paper and hybridized with nick-translated Ad5 DNA. (a) Wild-type Ad5; infection performed at 37°C. The same result was obtained with the wild type at 32°C. (b) Temperature-sensitive mutants grown at permissive (32°C) and nonpermissive (37°C) temperatures.*

TABLE 1. *Fractional yield of defective viral DNAs in Ad5-infected CV-1 cells labeled at various times after infection*

Time of labeling (h)	Fraction of total label shorter than genome length (10 to 95% of complete genome) in expt:			
	1	2	3	4[a]
26–42	0.40	0.27	0.21, 0.25	
18–36		0.25		0.41, 0.45, 0.39
6–24[b]	0.35			

[a] Two different plaque-purified stocks and a high-multiplicity stock were used to infect separate plates at identical multiplicities of infection (see text).

[b] Label incorporated into cellular DNA was omitted from the calculation.

TABLE 2. *Size distribution of labeled viral DNAs in Ad5-infected CV-1 cells*

Size range[a]	cpm	% of total counts	Estimated fraction of molecules[b]
Full length (>0.95)	4,460	60	0.38
0.40–0.95	1,630	22	0.23
0.30–0.40	540	7.3	0.12
0.17–0.30	570	8.4	0.19
0.13–0.17	150	3.2	0.09

[a] Restriction fragments A through D of Ad2 digested with *Bam*HI were used as size markers.

[b] The number of total counts in each region was divided by the average fractional length of the DNA in that region to yield an estimate of the number of molecules represented by the radioactive counts.

Plaquing efficiency of Ad5 and Ad2 on CV-1 and HeLa cells. The yield of virus particles from Ad2 infection of monkey cells is about 1,000-fold lower than the yield from human cells (32). Grodzicker et al. (14) reported that the plaquing efficiency on monkey cells is likewise 1,000-fold reduced and thus is a useful measure of comparative virus production. In this study, Ad2 had a 1,000-fold-lower plaquing efficiency, and Ad5 plaquing was reduced 10,000-fold on CV-1 cells and BSC-1 cells, relative to HeLa cells.

Effect of extensive plaque purification on yield of subgenomic DNAs. If subgenomic DNA fragments are generated by replication errors (4) and are themselves replicated, then the apparent serotype-specific distribution of predominant bands could arise from initial random generation of lengths, the predominant size classes being perpetuated through contamination of wild-type virions with seed amounts of defective DNA-containing particles. Defective RNAs synthesized by vesicular stomatitis virus are generated in this way, and an apparent strain specificity can in fact be lost by extensive plaque purification (16, 27, 34). The observation that Ad5 generated subgenomic DNAs in monkey cells but not in premissive cells allowed rigorous testing of the proposal that in the case of adenovirus-defective genomes the array of predominant sizes is genetically determined.

The high-titer stock of Ad5 was diluted and plaqued on HeLa cells. Several plaques were picked and further purified by six successive rounds of plaque purification as described above. Stocks prepared from plaques from the last round were used to infect CV-1 cells at a multiplicity of 2 PFU per cell.

The results of infection by two such plaque-purified stocks were compared with the results from a dilution of the original stock which had not been plaque purified. The yields of species of DNA of various sizes from CV-1 cells infected with these three stocks were nearly identical (Table 1, experiment 4). The distribution of subgenomic pieces generated by plaque-purified stocks was indistinguishable from that shown in Fig. 1 and 2 for Ad5.

Generation of subgenomic DNAs by temperature-sensitive mutants of Ad5 grown in monkey cells. A large number of temperature-sensitive mutants of Ad5 have been isolated and characterized. Three mutants which contain lesions in late functions and have been shown to synthesize viral DNA efficiently at the nonpermissive temperature (35, 40) were chosen to test for the generation of subgenomic DNAs. One of these (H5*ts*19) forms assembly intermediates at the nonpermissive temperature, whereas the other two do not (7). Some processing of core protein precursor pVII to its mature form (polypeptide VII) is seen in cells infected with H5*ts*19 at the nonpermissive temperature, whereas H5*ts*18 and H5*ts*1 synthesize pVII but show no processing (7, 11; unpublished data).

BSC-1 cells were infected with H5*ts*1, H5*ts*18, or H5*ts*19 at a multiplicity of 10 PFU per cell at either a permissive (32°C) or a nonpermissive (38.5°C) temperature. Figure 2b shows the viral DNA species generated during replication of these mutants at these two temperatures as detected by Southern hybridization. Photographic negatives were traced with a densitometer, and the area under the curves was determined. Figure 3a and b shows representative tracings, and Table 3 is a summary of the results. In three separate sets of infections, the mutants produced a relatively higher fraction of subgenomic DNA at the nonpermissive temperature than at the permissive temperature.

The fractional yields of subgenomic DNAs in cells infected with temperature-sensitive mutants are the highest observed under any conditions, although the difference between the

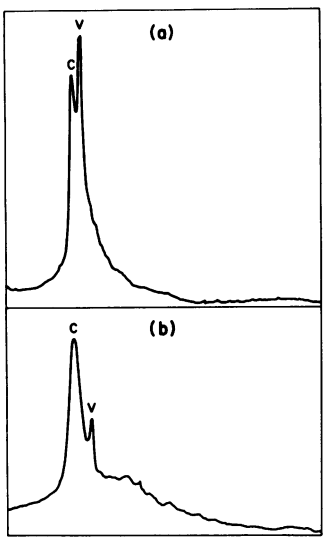

FIG. 3. *Quantitation of the distribution of sizes of DNA extracted from BSC-1 cells infected with H5ts1. DNA was extracted and electrophoresed; the gels were stained with ethidium bromide and photographed. Photographic negatives were traced with a Joyce-Lobel microdensitometer. (a) Infection at 32°C; (b) infection at 39°C. C, Cellular DNA; V, full-length viral DNA.*

TABLE 3. *Fractional yield of defective viral DNAs in BSC-1 cells infected with temperature-sensitive mutants of Ad5*

Virus	Temp (°C)	Fraction of viral DNA which is subgenomic[a]
Ad5	37	0.37
H5*ts*1[b]	39	0.69
	32	0.40
H5*ts*18	39	0.75
	32	0.30
H5*ts*19[b]	39	0.65
	32	0.35

[a] The relative amounts of full-length and subgenomic viral DNA were determined from measurements of the areas under curves generated from gel tracings (Fig. 3).

[b] The average of three separate infections and DNA preparations at each temperature is given.

amounts of subgenomic viral DNAs produced at the two temperatures is no greater than the variation that was seen from experiment to experiment with wild-type virus at one temperature. Wild-type Ad5 showed no temperature-dependent variation in the fractional amount of small DNAs generated. None of these mutants produced any detectable subgenomic species of DNA in HeLa cells at either temperature.

H5*ts*125 is a mutant of Ad5 which is blocked in DNA replication at the nonpermissive temperature (8). Investigation of this mutant showed that at the permissive temperature it too produced a normal array of Ad5 subgenomic species. As expected, there was no viral DNA made at the nonpermissive temperature. There is progressively less viral DNA synthesized as cells are infected with the mutant at temperatures above the permssive temperature, but the relative yield of subgenomic species does not change; rather, all species decrease proportionately.

Purification and restriction analysis of low-molecular-weight viral DNA produced in monkey cells. ^{32}P-labeled incomplete viral DNAs from Ad5-infected CV-1 cells were separated from complete viral DNA on sucrose gradients. Figure 4 shows such a gradient; fractions well separated from the complete viral DNA peak were pooled, precipitated, and analyzed with restriction endonuclease *Hin*dIII. The restriction fragments resulting from digestion of complete viral DNA and of the pooled incomplete DNAs are shown, with the Ad5 *Hin*dIII restriction map, in Fig. 5. In the digestion of incomplete DNA, the fragments from both ends of the full-length genome were present, but the fragments from internal regions appeared to be missing.

Production of subgenomic DNAs in fully permissive adenovirus infections of monkey cells. Since the fraction of viral DNA synthesis which results in subgenomic species is, at most, 40%, this phenomenon alone cannot account for the low yield of infectious virus in Ad5-infected monkey cells. To determine whether there is any correlation between production of subgenomic DNAs in Ad5-infected monkey cells and the semipermissive nature of the infection,

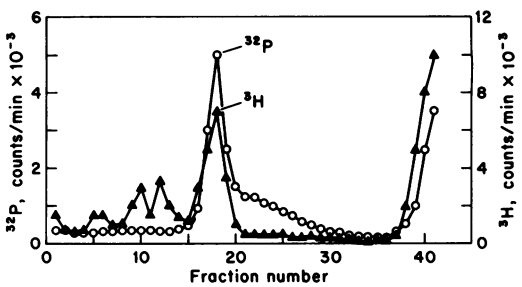

FIG. 4. *Sedimentation of labeled DNA from cells infected with ^{32}P-labeled DNA from Ad5-infected CV-1 cells or with ^{3}H-labeled DNA from Ad5-infected HeLa cells. DNAs were centrifuged through 5 to 20% neutral sucrose as described in the text.*

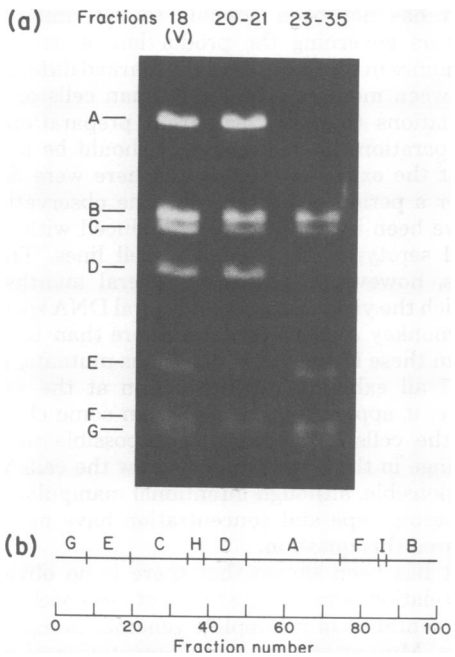

FIG. 5. *Restriction endonuclease digestion of pooled fractions of intracellular DNA from Ad5-infected CV-1 cells.* ^{32}*P-labeled DNA was precipitated from appropriate fractions from a sucrose gradient (Fig. 4), suspended, and digested with restriction endonuclease HindIII. (a) DNA was electrophoresed through 1.4% agarose for 12 h at 40 V; the gel was dried and autoradiographed. (b) Restriction map of Ad5 DNA digested with HindIII. V, Full-length viral DNA.*

three permissive infections of these cells with adenoviruses were investigated.

First, the production of subgenomic species by Ad5 HR404, a host range mutant of Ad5 selected for normal growth on monkey cells, was measured. Second, cells were coinfected with simian virus 40 and Ad5, in which the block to production of infectious Ad5 is fully relieved by a complementing simian virus 40 function. Virus yields in both cases were equivalent to yields in HeLa cells. The fractional yield of subgenomic DNAs and the array of sizes of viral DNAs, as revealed by blotting (Fig. 2), were the same as those in the semipermissive infections by uncomplemented wild-type virus.

The third piece of evidence which indicates that the semipermissive infection is not directly related to the production of subgenomic species in monkey cells comes from studies of an adenovirus with a different host range. SA7 is 1 of 18 adenoviruses which have been isolated from monkeys and partially characterized (3). CV-1 and BSC-1 cells are fully permissive for this virus, producing 10^3 or more infectious particles per infected cell. The yield of this virus is one or two orders of magnitude lower in HeLa cells than in monkey cells. Viral DNA synthesis is reduced but easily detectable. Figure 6 shows the array of subgenomic species produced in SA7-infected CV-1 cells. In HeLa cells, only full-length viral DNA was detected, even by sensitive techniques of blotting and hybridization. In these three cases, infected monkey cells yielded a high proportion of aberrant DNAs, although the virus seemed to undergo a fully productive growth cycle.

DISCUSSION

It has been shown that a substantial proportion of the viral DNA synthesized after infection of monkey cells with adenovirus types 2 and 5 is shorter than the full-length viral genome. Studies of intracellular viral DNA in Ad3-infected HeLa cells have led us to conclude that the packaging of DNA into particles does not govern the selection of certain sizes of incomplete DNAs. The synthesis of specific species of unpackaged subgenomic DNAs in Ad5-infected monkey cells supports these conclusions. Less than 0.01% of the DNA made in Ad2- and Ad5-infected CV-1 cells becomes associated with virus particles.

The size distribution of DNA from incomplete particles (4) suggests that each adenovirus serotype generates an array of subgenomic DNAs which is characteristic and reproducible. In Fig.

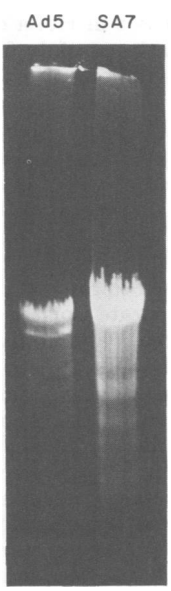

FIG. 6. *Comparison of electrophoretic patterns of subgenomic species of Ad5 and SA7 generated in BSC-1 cells. Preparation was as described for Fig. 1, with 5 μg of DNA per slot.*

6, this point is illustrated by a comparison of the Ad5 pattern with the SA7 pattern. Such a specificity could arise in two ways: either the wild-type genome is predisposed to generate a particular set of size classes of aberrant DNA or each stock used to infect contains seed amounts of defective genomes randomly generated in an early passage after plaque purification. The latter is the case for vesicular stomatitis virus, which generates defective particles containing subgenomic RNAs with inverted terminal repetitions (23, 28, 29). For many years, it was thought that different vesicular stomatitis virus strains gave rise to genetically determined classes of defective particles (27, 34). It has now been demonstrated by Holland et al. (16) that, after careful serial cloning of vesicular stomatitis virus, a random and unpredictable array of defective particles is generated; the particles then breed true in subsequent passages and are enriched.

Several characteristics of adenovirus defective particles and subgenomic DNAs suggest that there is genetic determination of size classes, rather than the random generation and enrichment shown by vesicular stomatitis virus. Production of defective particles or of intracellular subgenomic DNAs by Ad3 does not depend on the multiplicity of infection, and plaque-purified stocks give the same fractional yield of defective particles as do late-passage stocks prepared by high-multiplicity infection (4). The fact that Ad5 produces short specific size classes of DNA in monkey cells but not in HeLa cells makes this system particularly useful for determining whether separate individual genomes may generate a pool of short DNAs with a different pattern of sizes. The sequential plaque purifications described were performed as extreme measures to assure that the isolates were completely ndependent. It is unlikely that there is any contamination with defective genomes since none were detectable initially, and this purification protocol is designed to remove them, if present.

When these separate isolates were then used to infect CV-1 cells, after only one round of replication in HeLa cells, a distribution of defective viral genomes which is indistinguishable from that generated by the original high-multiplicity stock was observed. I feel that it has been conclusively shown that the selection of predominant sizes is somehow determined by the wild-type genome, perhaps by particular aspects of its sequence (4). Four temperature-sensitive mutants of Ad5 which had been independently isolated and maintained in different laboratories also show identical distributions of subgenomic DNAs, supporting the results of the plaque purification experiment.

It has not been possible to determine the factors governing the production of defective genomes in terms of either the marked difference between monkey cells and human cells or the variations that are seen from preparation to preparation. In this regard, it should be noted that the experiments reported here were done over a period of 2 years, and the observations have been independently reproduced with several serotypes, mutants, and cell lines. There was, however, a period of several months in which the yield of subgenomic viral DNA species in monkey cells was reduced more than 10-fold from these levels. Ad5, its various mutants, and SA7 all exhibited this reduction at the same time; it apparently resulted from some change in the cells or medium. It is possible that a change in the serum used to grow the cells was responsible, although intentional manipulations of serum type and concentration have not answered this question.

It has been shown that there is no obvious correlation between the levels of virus yield and the synthesis of incomplete genomes in monkey cells. Mutant studies, complementation of Ad5 by simian virus 40, and the fact that SA7 also produces a large fraction of defective genomes in these cells argue against such a correlation. As previously noted, the fraction of viral genomes which are aberrant does not account for the low yield of infectious particles in monkey cells infected with human adenoviruses.

The large yield of subgenomic viral DNAs in cells infected with temperature-sensitive mutants at nonpermissive temperatures, relative to the yield at the permissive temperature, suggests that some late viral functions are important in modulating the events which lead to aberrant DNA production. The three late mutants examined packaged DNA to different extents; yet all showed an increase in subgenomic DNAs at the higher temperature. As reviewed above, the late function(s) involved in host range restriction seems to have no direct effect on this phenomenon. It seems likely that a balance between two or more synthetic processes in infected cells is responsible for the variation from experiment to experiment and for the consistent cell-type-dependent differences. Further work with other mutants, and perhaps with a variety of inhibitors of DNA or protein synthesis (17), may prove useful in defining the parameters involved.

ACKNOWLEDGMENTS

I thank Gale Garcia and Taffy Mullenbach for excellent technical assistance, Nick Story for preparing the figures, Leslie Stevens for critical reading of the manuscript, and Barbara Kellogg for typing and proofreading. John Holland has provided invaluable advice.

This investigation was supported by Public Health Service grant CA 19058 from the National Cancer Institute and by Biomedical Sciences Support grant RR07006-11 from the General Research Support Branch, Division of Research Resources, National Institutes of Health.

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