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Transformation of Rat Cells by *cyt* Mutants of Adenovirus Type 12 and Mutants of Adenovirus Type 5

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Several mutants with much reduced oncogenicity (spontaneous mutants H12 *cyt* 52 and H12 *cyt* 70 and UV-induced mutants H12 *cyt* 61, H12 *cyt* 62, and H12 *cyt* 68) of the highly oncogenic adenovirus type 12 (Ad12) were studied for their ability to transform primary baby rat kidney cells. Four of the mutants showed much reduced capacity to transform cells in vitro, while H12 *cyt* 61 transformed cells as efficiently as the wild-type virus. Viral gene expression in several cell lines established from cultures infected by *cyt* mutants was studied, and it was found that viral sequences belonging to the left 16% of Ad12 were always transcribed. These results suggest that the function of the transformed state is not defective in the *cyt* mutants studied. Heterotypic complementation studies showed that the defect(s) in a *cyt* mutant can be corrected by an Ad7 function. Ad5 *dl* 313, with a deletion between 3.5 and 10.5 map units, transformed rat cells only at high multiplicity. These results suggest that the region E1B of adenoviruses may be required for efficient transformation of rat cells.

Human adenovirus type 12 (Ad12) is highly oncogenic in newborn hamsters and can transform rodent cells in tissue culture. It has been established by DNA transfection experiments that region E1 of adenoviruses is sufficient to establish transformed cells in vitro (8, 24, 31, 32). DNA fragments containing only E1A regions can induce partial or incomplete transformants (3, 14, 34). However, adenovirus mutants with a defect(s) in either region E1A or E1B have a transforming capacity different from that of the wild-type virus. Host range mutants of Ad5 belonging to two complementation groups have been isolated (9). Group I mutants are defective in inducing stable transformants while group II mutants show no transformation ability. Jones and Shenk (16) have isolated a group of host range deletion mutants of Ad5. Two mutants, *dl*312 and *dl*313, which have deletions from 1.5 to 4.5 and 3.5 to 10.5 map units, respectively, are transformation negative for rat embryo and rat brain cells. Recently, it has been shown that Ad5 *dl*313 induced incomplete transformation of an established rat cell line 3Y1 (33). An Ad5 mutant expressing E1B, but not E1A, cannot transform primary rat cells (36). Therefore, it is possible that cellular transformation by adenovirus may require two separate functions.

A group of *cyt* mutants has been isolated (39) from the highly oncogenic human Ad12 (group A). The *cyt* mutants produce large clear plaques, whereas the wild-type virus gives small fuzzy-edged plaques on human embryonic kidney (HEK) cells. During lytic infection, these mutants induce extensive cellular destruction (atypical adenovirus cytopathic effect) (39) as well as DNA degradation (4). The wild-type cytopathic effect is dominant over the *cyt* mutant cytopathic effect. Furthermore, all *cyt* mutants studied belong to a single complementation group. In general, these *cyt* mutants are much less tumorigenic in newborn hamsters. Takemori et al. (38, 39) were unable to transform hamster kidney cells with several *cyt* mutants using the method described by McBride and Wiener (25). Revertants have been isolated from some spontaneous but not from the UV-induced *cyt* mutants. These revertants recovered the plaque morphology, the cytopathic effect, and the tumorigenicity of the wild-type virus (40). It has been hypothesized that a single gene is responsible for both cellular destruction and tumorigenicity. We have recently shown that the *cyt* mutation affects functions encoded in region E1B of Ad12 (18).

In this report, we show that several *cyt* mutants are defective in transformation. Complementation studies on transformation between a *cyt* mutant and the weakly oncogenic Ad7 suggest that Ad7 can complement the defect(s) in the *cyt* mutants. We also found that Ad5 *dl* 313 transformed primary rat kidney cells only at high multiplicites of infection. Thus, the viral gene(s) responsible for efficient transformation of rat

cells may reside in the E1B region of the adenovirus genome.

MATERIALS AND METHODS

Cells and viruses. Human embryonic kidney cells (HEK) and KB cells were maintained as monolayer cultures using minimal essential medium (MEM) plus 10% fetal calf serum (FCS) or 10% calf serum. The 293 cell line obtained from Graham (10) was maintained in MEM (Joklik modified) with 5% horse serum. Suspension cultures of KB cells in MEM (Joklik modified) plus 5% horse serum were also used for growth of some viruses. Primary rat kidney cells were prepared by trypsinization of finely minced kidney cells from 7- to 12-day-old Chester Beatty rats in citrate saline (1% KCl, 0.44% sodium citrate). The cells were then seeded into 60-mm plastic dishes and cultured in αMEM plus 10% FCS. Wild-type Ad12 strains Huie and 1131, all five *cyt* mutants (isolated from strain 1131), and a revertant were obtained from Takemori and have been described (39). H12 *cyt* 61, H12 *cyt* 62, and H12 *cyt* 68 (UV-induced isolates) and H12 *cyt* 52 and H12 *cyt* 70 (spontaneous isolates) were originally designated as *cyt* 133, *cyt* 135, *cyt* 7, *cyt* 129, and *cyt* 191, respectively. Crude virus stocks were used to infect HEK cells, and the lysates were subsequently used to infect KB cells using monolayer cultures (5). Ad5 wild type and group II host range mutant (*hr6*) stocks were obtained from F. Graham and were propagated in KB cells grown in suspension and 293 cells, respectively. Ad5 *dl*312 and Ad5 *dl*313 were obtained from T. Shenk and have been described previously (16). They were propagated in 293 cells. Virions purified by CsCl gradient centrifugation were used throughout these experiments.

Transformation and tumorigenicity assays. Primary rat kidney cell cultures at 60 to 80% confluency were washed with adsorption medium (suspension culture medium plus 1% FCS) and then infected with 0.2 ml of virus suspension. After 90 to 120 min of adsorption at 37°C, the cells were fed with αMEM plus 10% FCS (Amour Chemicals). After 2 to 3 days, the medium was changed to MEM without calcium (Joklik modified) plus 5% horse serum. The culture medium was replaced with fresh medium twice weekly, and the cells were fixed with Carnoy fixative and stained with Giemsa on the 23rd day after infection for determination of foci. To establish cell lines, well-isolated colonies were picked and separately grown up in MEM without calcium plus 5% horse serum.

For tumorigenicity studies, weanling Chester Beatty rats (3 to 4 weeks old) were injected subcutaneously with 10^6 transformed cells per animal and examined weekly for tumors.

Preparation of cellular DNA and viral DNA, restriction endonuclease digestion, and gel electrophoresis. To prepare cellular DNA, cells grown on plastic dishes were washed with phosphate-buffered saline without Ca^{2+} or Mg^{2+} lysed with 1% sodium dodecyl sulfate in 0.01 M Tris (pH 7.4), and digested with pronase (200 μg/ml) overnight. The lysate was extracted three times with phenol. The nucleic acids were precipitated by ethanol and treated with RNase. The organic extractions were repeated, and the DNA was dialyzed extensively against 0.01 M Tris (pH 7.9). Viral DNA was extracted from purified virions.

Restriction endonucleases *Hin*dIII, *Kpn*I, and *Sac*I were purchased from New England Biolab, and the digestions were carried out for 2 to 3 h at 37°C with 1 to 3 U per μg of DNA under assay conditions suggested by the manufacturer. DNA fragments produced by restriction endonucleases were separated in 1% agarose vertical or horizontal gels by electrophoresis at 2 V/cm for 9 to 11 h (22).

Isolation and in vitro labeling of Ad5 viral DNA fragments. pBR-322 plasmid DNA containing the left 10.5% of the Ad5 genome was a gift from F. Graham, McMaster University. The DNA was digested with restriction endonuclease *Sac*I, and the resulting fragments were separated by agarose horizontal gels and recovered from the gel according to a published procedure (7). The DNA fragments were labeled in vitro by nick translation with [α-^{32}P]dCTP after the DNA was incubated with 1 ng of DNase I per μg of DNA for 15 min at 37°C (28, 43). *Sac*I cleaves Ad5 DNA at 5 and 10.5%, thus generating viral sequences corresponding to approximately early E1A and E1B regions.

Detection of viral DNA sequences in transformed cells. DNA extracted from cells transformed by Ad5 *dl*313 virions was cleaved with endonuclease *Kpn*I, separated by gel electrophoresis, transferred to nitrocellulose filters (37, 42), and hybridized to Ad5 DNA fragments labeled with ^{32}P by nick translation. The hybridized radioactivity was detected by autoradiography with Kodak film XAR-5.

Detection of viral RNA. Transformed cells were labeled with [^{3}H]uridine at 50 μCi/ml for 1 to 2 h, and whole-cell RNA was extracted by the hot phenol method (23). After alcohol precipitation, the RNA was treated with DNase, reextracted with hot phenol, and dialyzed extensively against 0.1× SSC (0.15 M NaCl plus 0.015 M sodium citrate). [^{3}H]RNA (2×10^7 to 5×10^7 cpm) was used to hybridize to 2 μg of viral DNA fragments generated by restriction endonuclease *Hin*dIII according to procedures already described (35, 37).

Protein labeling and immunoprecipitation. Transformed cells grown in 150-cm plastic dishes were labeled in culture medium containing one-third of the unlabeled methionine in MEM plus 10% FCS and 200 μCi of [^{35}S]methionine (specific activity, 1,220 Ci/mmol) per dish for 14 h. The procedure for immunoprecipitation is similar to that described by Schauffhausen et al. (30). The cells were harvested and washed with phosphate-buffered saline and lysed with 1% Nonidet P-40–10% glycerol in 100 mM Tris (pH 8)–100 mM NaCl–1 mM $CaCl_2$–0.5 mM $MgCl_2$. The lysate was centrifuged, and an aliquot of the supernatant was mixed with antiserum C1 and protein A-Sepharose CL4B (Pharmacia Fine Chemicals, Inc.) and incubated at 5°C overnight with constant mixing. Antiserum C1 was from rats bearing tumors which were induced by cells transformed by the left 16% of Ad12 DNA (24). The Sepharose beads were washed repeatedly with a buffer containing 100 mM Tris (pH 8.2), 200 mM LiCl, and 1% 2-mercaptoethanol. The immune complex was eluted by boiling in elution buffer (5% sodium dodecyl sulfate, 5% 2-mercaptoethanol, 20% glycerol, 100 mM Tris [pH 8.0]). The immunoprecipitated proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel (15.2%) electrophoresis (17). ^{35}S-labeled proteins were detected by fluorography (19). ^{14}C-labeled molecular weight markers were purchased from Bethesda Research.

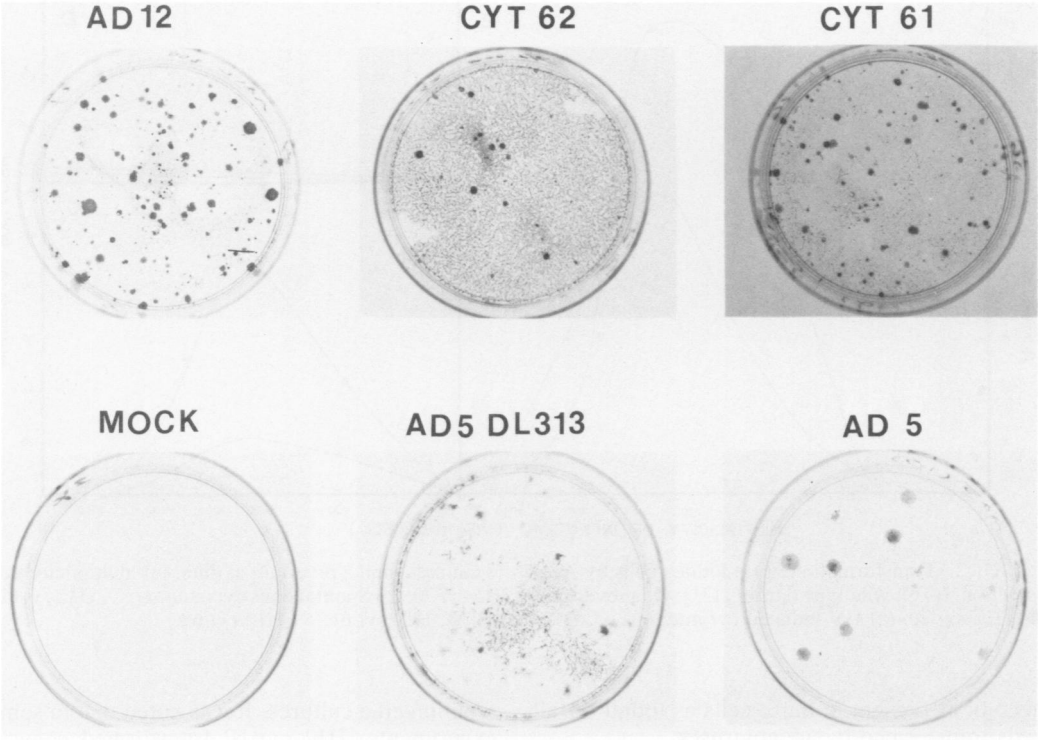

FIG. 1. Photographs of transformed foci induced by infection of primary rat kidney cells. Multiplicity of infection in virions per cell is as follows: Ad12 wild type, 800; *cyt* 62, 800; Ad5 *dl*313, 800; Ad5 wild type, 1.5.

RESULTS

Transformation by *cyt* mutants. Primary baby rat kidney cells were used to assay transformation by purified virions of Ad12 (strain 1131) and of *cyt* mutants. In this system, noninfected cultures had no cells left in the dishes after 3 weeks, while transformed foci appeared darkly stained (Fig. 1). The infected cultures, in particular those infected with mutant virus, sometimes show a background of nontransformed cells. When these cells are examined under the microscope, they do not have the typical morphology of transformed cells. Figure 2 shows the results of a typical transformation experiment. For the wild-type virus, the number of transformed foci was proportional to the number of virion input, reaching a maximum at 800 virions per cell (Fig. 2a). At higher multiplicities of infection, the number of foci declined, probably due to cell killing by the virus. Two *cyt* mutants (UV induced) (*cyt* 62 and 68; Fig. 2C) induced transformation at a lower frequency, with a similar dependency on multiplicity of infection. The maximum number of foci is about 10 to 15% of that produced by the wild-type Ad12. Two spontaneous *cyt* mutants (*cyt* 52 and *cyt* 70; Fig. 2b) induced transformed foci only at very high input multiplicities. On the other hand, H12 *cyt* 61 transformed rat kidney cells with a dose response similar to the wild-type Ad12 (Fig. 2c). A revertant of the nontransforming H12 *cyt* 70 recovered its transforming ability similar to that of the wild type as shown in Fig. 2a. These data indicate that the majority of the *cyt* mutants are defective in transformation.

Transcription of viral genes in cells transformed by *cyt* mutants. To ascertain whether the transformants in *cyt* mutant-infected cultures were induced by Ad12 virions, we studied viral gene transcription in some of these cell lines using the Southern technique. [^{3}H]RNA was hybridized to Ad12 DNA fragments generated by *Hin*dIII restriction endonuclease, and the radioactive hybrids were detected by fluorography. Figure 3 shows microdensitometer scans of the resulting autoradiographs. [^{3}H]RNA from all of the cell lines examined hybridized to fragments F and D, corresponding to the left 16% of the Ad12 genome. Three cell lines (52-C1, 61-C1, 61-C2) synthesized also viral RNA complementary to fragment E (the right-hand 10%). In addition [^{3}H]RNA from several transformed cell lines was hybridized to late regions of the Ad12 genome (fragments A and C). These results indicate that the left-hand end of the Ad12

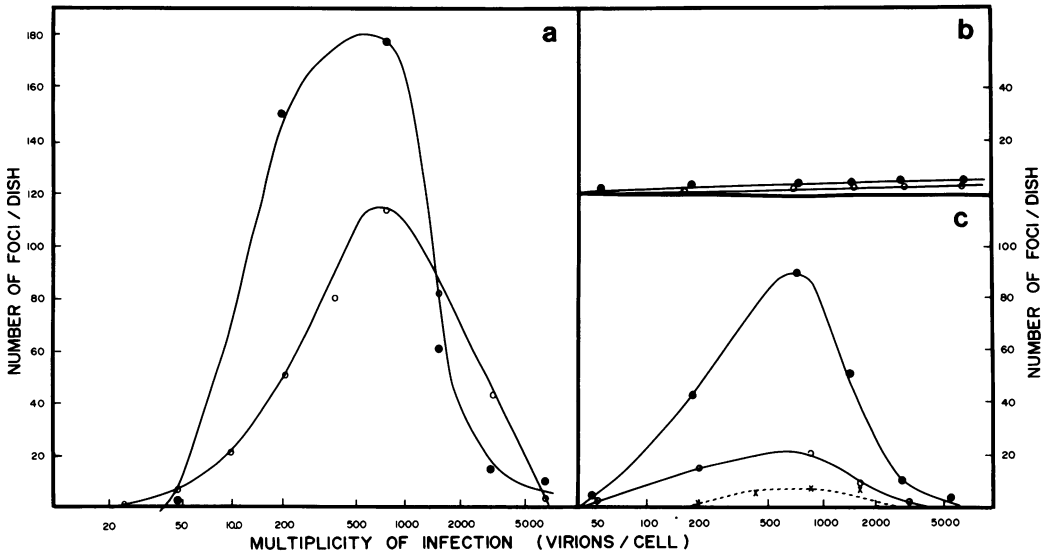

FIG. 2. Transformation of rat kidney cells by Ad12, *cyt* mutants, and a revertant at different multiplicities of infection. (a) ○, wild type (strain 1131); ●, a revertant of H12 *cyt* 70. (b) Spontaneous *cyt* mutants. ○, H12 *cyt* 52; ●, H12 *cyt* 70. (c) UV-induced *cyt* mutants. ○, H12 *cyt* 62; ●, H12 *cyt* 61; ×, H12 *cyt* 68.

genome is present in these cells as found for all cells transformed by adenoviruses.

Complementation studies in transformation by different *cyt* mutants. *cyt* mutants are thought to be produced due to mutations in a single cistron (40). However, these mutants are heterogeneous with respect to the ability to grow in a subline of KB cells, and wild-type recombinants were isolated from cells infected by pairs of *cyt* mutants (38, 40). Experiments were carried out to examine the possibility of complementation of transformation by some of the *cyt* mutants. Table 1 shows that coinfection of rat cells by H12 *cyt* 62 and H12 *cyt* 70 resulted in a higher number of transformants than the sum of transformants induced by these mutants separately. Similar results were obtained when H12 *cyt* 52 and H12 *cyt* 70 were used. However, the maximum number of transformants resulting from coinfection of cells was only about 10% of that from wild-type-infected cultures. It was noted that in some experiments, H12 *cyt* 62 transformed cells at about 10% of the level of the wild-type virus (see Fig. 2). Thus, no definitive complementation could be demonstrated. The weak complementation in transformation may be due to intragenic complementation as in the case for lytic infection (38).

Heterotypic complementation in transformation. Ad7, a weakly oncogenic adenovirus, can cooperate in tumor induction in hamsters (39). Both tumor antigens were detected in a line of tumor cells cultured from a cooperation experiment, and most of the tumor-bearing hamster sera showed antibodies against Ad7 and Ad12 tumor antigens. However, it is not clear from these in vivo studies how the viruses cooperated in giving rise to tumors. We tested the ability of Ad7 to complement the H12 *cyt* 70 mutant for transformation. Transformed foci were ob-

TABLE 1. Complementation of Ad12 *cyt* mutants in the transformation of baby rat kidney cells

Ad12 *cyt* mutant	Virions per cell	No. of transformed foci per dish[a]														
		H12 *cyt* 70					H12 *cyt* 62					H12 *cyt* 52				
		0[b]	50	200	800	1,600	0	50	200	800	1,600	0	50	200	800	1,600
H12 *cyt* 70	0	0	0	0	1	0.5	0	0	0.75	0.75		0	0	0	0.5	0.5
	400						0	21	19	19			16	22	26	
H12 *cyt* 62	0											0	0	0.75	0.75	2.75
	400			3	7	5							0.5	3.7	21	6

[a] The number represents the mean of four dishes.
[b] Number of virions per cell.

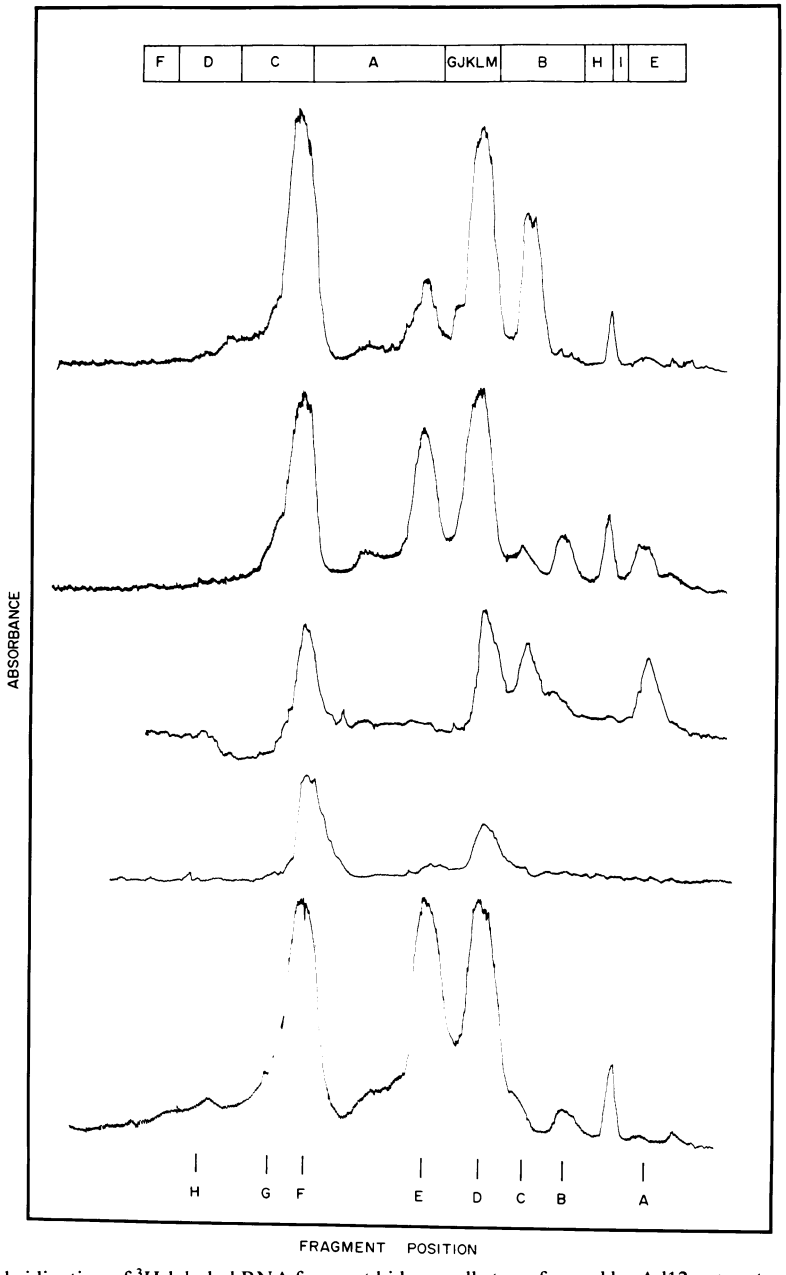

FIG. 3. Hybridization of ^{3}H-labeled RNA from rat kidney cells transformed by Ad12 *cyt* mutants to unlabeled *Hin*dIII fragments of Ad12 DNA. The hybridized radioactivity was detected by fluorography. Microdensitometer tracings of the resulting fluorographs are shown. The *Hin*dIII cleavage map of Ad12 (strain 1131) is also shown at the top. From top to bottom: [^{3}H]RNA isolated from cells transformed by H12 *cyt* 61 (clone 1), H12 *cyt* 61 (clone 2), H12 *cyt* 62 (clone 1), H12 *cyt* 70 (clone 4), and H12 *cyt* 52 (clone 1).

served in cultures infected by Ad7 alone. However, addition of H12 *cyt* 70 virions to the infection enhanced the transformation frequency (Table 2). These data indicate that a substantial number of transformants may be the result of *cyt* mutant infection. To test what fraction of the transformed foci has the phenotype of Ad12-transformed cells, we took advantage of the fact that Ad12-transformed cells induce tumors in immunocompetent rats, while those transformed

TABLE 2. Transformation of rat kidney cells by Ad7, H12 *cyt* 70, or a mixture of the two viruses

Virus[a]	No. of foci per dish[b]	
	Expt 1	Expt 2
Ad7	9	30
H12 *cyt* 70	2	0
Ad7 plus H12 *cyt* 70	22	70

[a] A total of 400 virions of each virus type was used per cell.

[b] The number represents the mean of four dishes.

by Ad7 do not. Six cell lines were established from coinfected cultures in different transformation experiments and tested for tumorigenicity. Four of these cell lines induced tumors while all four Ad7-transformed cell lines did not (Table 3).

To detect the viral genes expressed in these transformants, radioactive RNA from two tumorigenic and two non-tumorigenic transformed cell lines established from the coinfected cultures was hybridized to Ad12 DNA fragments generated by *Hin*dIII. [^{3}H]RNA from two tumorigenic cell lines hybridized to the left 16% of the Ad12 genome (fragments F and D) (Fig. 4, bottom two tracings) but did not hybridize to Ad7 DNA (data not shown). The RNA synthesized by the non-tumorigenic lines did not hybridize to the left 16% but hybridized to *Hin*dIII-B fragment of Ad12 (Fig. 4, top two tracings), which corresponds to early region III (27, 35). The RNA from these two cell lines also hybridized to fragments *Bam*HI-H and -I of Ad7 DNA, the left 2 to 9.6% (41) (data not shown). [^{3}H]RNA from cells transformed by Ad7 alone hybridized to the same Ad7 DNA fragments (data not shown).

To demonstrate that Ad12 region E1 proteins were synthesized in these tumorigenic cells, the transformed cells were labeled with [^{35}S]methionine and immunoprecipitated with antiserum C1. Figure 5 shows the immunoprecipitated proteins fractionated by polyacrylamide gel electrophoresis. A 58,000-molecular-weight (58K) polypeptide was precipitated from both the CT7-3 and CT7-4 lysate by the antiserum C1, but was not precipitated by nonimmune rat serum. In addition, a 19K polypeptide was also detected by antiserum C1 in the CT7-3 lysate. These polypeptides could also be detected in Ad12-infected cell lysates by immunoprecipitation by antiserum C1 (H. Galet, unpublished data). Published reports suggest that these are probably E1B products (1, 15). These tumor antigens cannot be coded for by the Ad7 genome since no Ad7 E1B RNA was found in these cell lines.

Data obtained so far indicate that the transformation frequency by a *cyt* mutant can be greatly increased by coinfection with Ad7, suggesting that the viral genes responsible for the transformed phenotype are not defective in this mutant.

Transformation by Ad5 host range and deletion mutants. We have shown that the *cyt* mutation affects the E1B function(s) (18). It is possible that Ad5 host range and deletion mutants which have defects in region E1B (12, 16) may be capable of transforming cells in vitro under appropriate conditions. Rat kidney cells were infected with Ad5 group II host range mutant, *hr*6, and with Ad5 *dl*312 and Ad5 *dl*313, which have deletions from 1.5 to 4.5 and 3.5 to 10.5 map units, respectively. The number of foci per three dishes for different multiplicities of infection are shown in Fig. 6. It can be seen that cultures infected with wild-type Ad5 gave the maximum number of foci at 1.5 virions per cell. No foci were observed when cells were infected with either Ad5 *dl*312 or host range mutant *hr*6 in agreement with published results (9, 16). However, Ad5 *dl*313 transformed rat cells at high multiplicities, reaching a maximum value of 85 foci at 800 virions per cell. These foci can be established as permanent cell lines as easily as foci transformed by wild-type Ad5.

To show that these foci were the results of transformation by Ad5 *dl*313, we examined the Ad5 DNA sequences in four established cell lines. The cellular DNA was cleaved with endonuclease *Kpn*I and electrophoresed, blotted, and hybridized to ^{32}P-labeled Ad5 E1B DNA (5 to

TABLE 3. Tumorigenicity of rat kidney cells transformed either with Ad7 singly or a combination of Ad7 and an Ad12 *cyt* mutant (H12 *cyt* 70)

Cell line	Isolated from cultures infected with:	Fraction of animals with tumors
cyt 70-7-C1	Ad7 and H12 *cyt* 70	0/10
cyt 70-7-C2		0/10
cyt 70-7-C3		17/19
cyt 70-7-C4		9/10
cyt 70-7-C5		8/9
cyt 70-7-C6		10/10
Ad7-C1	Ad7	0.11
Ad7-C2		0/10
Ad7-C4		0/10
Ad7-C6		0/9
PC1	Ad12	11/11
PC2		7/7
PC3		7/8
cyt 70-C1	H12 *cyt* 70	15/20
cyt 70-C3		10/19
cyt 70-C4		15/20

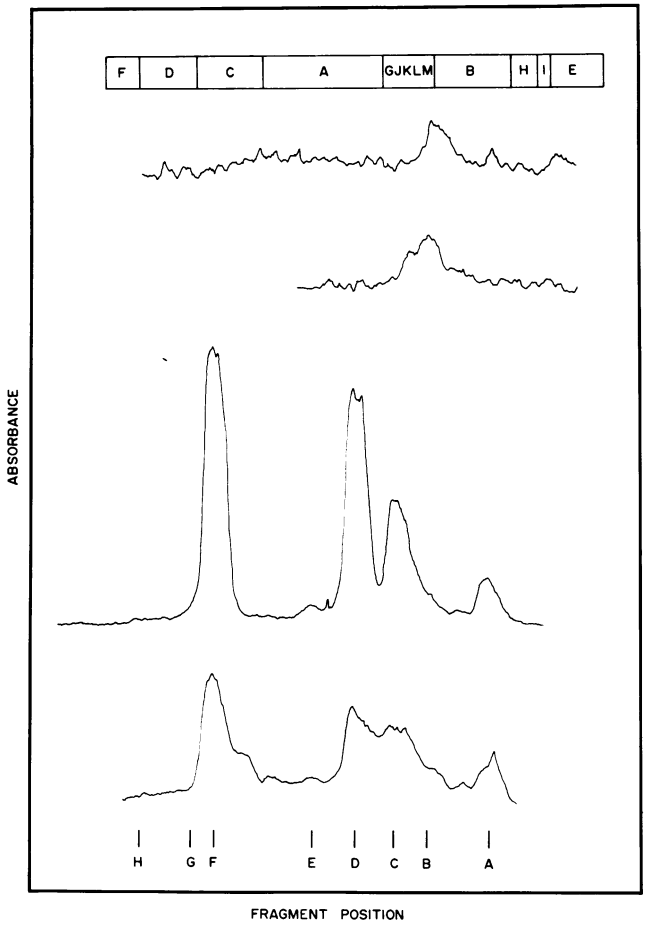

FIG. 4. Hybridization of ^{3}H-labeled RNA from transformed rat kidney cells, isolated from cultures coinfected with Ad12 *cyt* mutant (H12 *cyt* 70) and Ad7 wild type virions, to *Hin*dIII fragments of Ad12 DNA. Details are as in Fig. 3. From top to bottom: [^{3}H]RNA isolated from non-tumorigenic cell lines CT7-1 and CT7-2 and from tumorigenic cell lines CT7-3 and CT7-4.

10.5% of Ad5). The autoradiogram resulting from such an experiment is shown in Fig. 7b. This probe hybridized to *Kpn*I-A fragment and a fragment having a size of 2.8% of the Ad5 genome (similar in size to fragment J) when Ad5 *dl*312 DNA was blotted (lane 1). The latter fragment corresponds to fragment H with the 3.5% deletion. When 1 ng of Ad5 *dl*313 DNA (corresponding to about 10 copies per cell) was hybridized to this probe, the autoradiogram shows a faint band (lane 2) corresponding to a fragment similar to fragment A in size. This corresponds to the fusion of fragments A and H minus the deletion. This low level of hybridization is due to the fact that there are eight base pairs in the probe not deleted in Ad5 *dl*313 DNA. It can be seen that the E1B region can be readily detected in a cell line transformed by wild-type Ad5 (lane 3) but not detected in four cell lines transformed by Ad5 *dl*313 (lanes 4, 5, 6, and 8). When ^{32}P-labeled Ad5 E1 DNA sequences were used as probes, the DNA from all five transformed cell lines showed positive hybridization (Fig. 7a, lanes 3, 4, 5, 6, and 8). Hybridization of this probe to DNA from Ad5 *dl*312 and Ad5 *dl*313 demonstrated that it contains only the E1 region of Ad5 DNA. Under our experimental conditions, we could detect as little as one copy of the Ad5 E1B region per transformed cell. It should be noted that the Ad5 E1 probe hybridized to transformed cell DNA with very large molecular weights. This is probably due to incomplete digestion of cell DNA by *Kpn*I enzyme. In any case, data do show that these cells contain Ad5 DNA corresponding to region E1A but not to E1B.

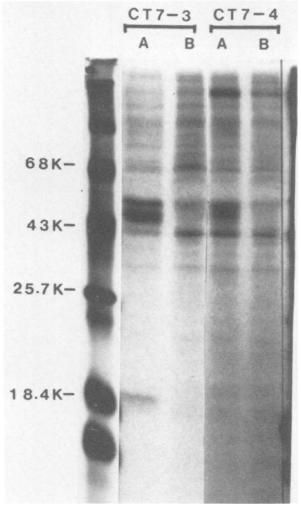

FIG. 5. Polyacrylamide gel electrophoresis of [^{35}S]methionine-labeled proteins from two tumorigenic cell lines (CT7-3 and CT7-4) established from cultures coinfected with Ad7 and H12 *cyt* 70. These proteins have been immunoprecipitated by antiserum C1 (lanes A) and normal rat serum (lanes B).

DISCUSSION

Takemori et al. isolated a group of mutants from the highly oncogenic Ad12 (39). These mutants exhibited a cytocidal phenotype during lytic infection and a reduced tumorigenicity in newborn hamsters. We found that four of the *cyt* mutants (H12 *cyt* 52, H12 *cyt* 62, H12 *cyt* 68, and H12 *cyt* 70) have much lower transforming ability. The defect in transformation appears to be heterogeneous; some mutants retain a reasonable capacity to transform cells while others may not transform at all (H12 *cyt* 70 and H12 *cyt* 52). The small numbers of transformants induced by the latter two mutants at a high multiplicity of infection may be due to revertants in the virus preparations. Their reduced turmorigenicity in vivo appears to coincide with the limited ability to transform cells in vitro. The fifth mutant studied, H12 *cyt* 61, had essentially normal transformation capacity (Fig. 2) in spite of its cytocidal phenotype: clear plaque morphology, low virus yield (unpublished observation), and degraded newly synthesized DNA in infected KB cells (5, 18). It was noted that another *cyt* mutant, H12 *cyt* 66, has been shown to be highly tumorigenic in hamsters (39). Thus, different gene products may be responsible for the functions of efficient oncogenic transformation and of cellular destruction. Although *cyt* mutants belong to a single complementation group and we have shown that the *cyt* mutation is mapped in region E1B (18, 40), it is still possible that different polypeptides encoded in this region are affected in H12 *cyt* 61 from those affected in the other *cyt* mutants. Alternatively, the mutation in H12 *cyt* 61 may map in a region outside of E1B. It should also be pointed out that H12 *cyt* 61 differs from other *cyt* mutants in that its DNA degradation function is dominant (18).

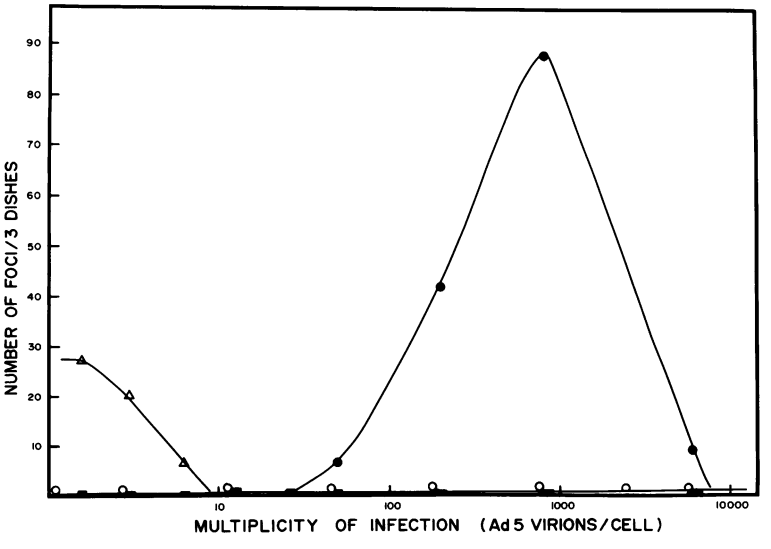

FIG. 6. Transformation of rat kidney cells by an Ad5 host range group II mutant and deletion mutants at different multiplicities of infection. Symbols: △, Ad5 wild type; ●, Ad5 *dl*313, ▬, Ad5 host range mutant (*hr*6); ○, Ad5 *dl*312.

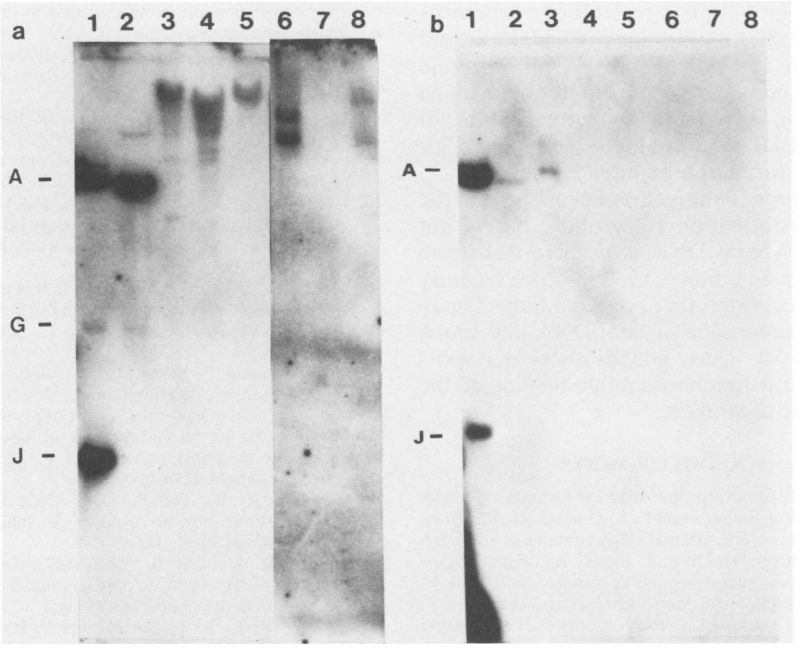

FIG. 7. Hybridization of ^{32}P-labeled Ad5 DNA fragments to cellular DNA isolated from cells transformed by wild-type Ad5 or from cells transformed by Ad5 *dl*313 virions. DNA from Ad5 *dl*312 or from Ad5 *dl*313 or cellular DNA was digested with *Kpn*I and transferred to nitrocellulose filters and hybridized to either ^{32}P-labeled DNA containing Ad5 DNA from 0 to 10.5% (a) or to ^{32}P-labeled pBR-322 DNA plus Ad5 DNA from 5 to 10.5% (b). Lane: 1, 1 ng of Ad5 *dl*312 DNA plus 15 μg of KB DNA; 2, 1 ng of Ad5 *dl*313 DNA plus 15 μg of KB DNA; 3, 15 μg of cell DNA from a cell line transformed with wild-type Ad5 virions; 4, 5, 6, and 8, 15 μg of cell DNA from four different cell lines transformed by Ad5 *dl*313 virions; 7, 15 μg of KB DNA.

We found that coinfection of H12 *cyt* 70 with Ad7 increased the number of transformants which have properties characteristic of Ad12-transformed cells rather than cells transformed by Ad7. These include tumor induction in immunocompetent animals, synthesis of RNA complementary to the left-hand end of the Ad12 genome, and production of Ad12 tumor antigens. In these experiments, the number of H12 *cyt* 70-transformed foci is 15 to 30 times more than expected if H12 *cyt* 70 and Ad7 were to transform rat cells independently. Thus Ad7 may have provided a function which is missing in H12 *cyt* 70. These data suggest that cooperation of Ad7 and *cyt* mutants in tumor induction (39) is at the process of cellular transformation rather than overcoming some host factors in the animal.

Ad5 *dl*313 has been shown to transform a rat cell line 3Y1 but not primary rat brain or embryo cells (16, 33). We have demonstrated that this mutant virus transformed cells at much higher multiplicities of infection (about 500 times) compared with wild-type Ad5 (Fig. 6). These transformed cells contain Ad5 E1A sequences, but not E1B, as expected. Although the transformed cells can be established as cell lines, they may have the same growth properties as those described as incomplete or partial transformants (14, 33). These results suggest that the Ad5 E1B region is not required for transformation (incomplete?) per se. Without the functional E1B products, the efficiency of transformation is very low. This factor may explain the difference between the earlier and the present results (16). In contrast to Ad5 *dl*313, the group II host range mutant (*hr*6) failed to transform cells even at a very high multiplicity of infection although the mutation has been mapped in region E1B (6.1 to 9 map units) (6). It is known that *hr*6 differs from Ad5 *dl*313 in that the former can replicate DNA in the nonpermissive human cells while Ad5 *dl*313 cannot (16, 20). Thus, it is possible that the group II host range mutants can transform cells at a high multiplicity of infection but that the transformants are killed because of the additional viral functions encoded in region E1B, which are missing in Ad5 *dl*313. The observation that both Ad12 and Ad5 at high multiplicities of infection inhibited transformation of rat cells lends support to this suggestion (see Fig. 2 and 6). At least three polypeptides (~14K, 15K, and

58K) have been assigned to E1B of adenovirus (29). Since both Ad5 *hr*6 and Ad5 *dl*313 do not synthesize the 58K polypeptide (21), this protein cannot be responsible for the different effects on transformation between the two viruses. It is not clear which E1B gene products are required for efficient transformation by adenoviruses.

Polyoma large T antigen is necessary for the efficient transformation by virions, but is not required when viral DNA is used to transform cells or to induce tumors (13, 26). More recently it has been suggested that polyoma large T may regulate the integration of viral DNA into transformed cells (2). Thus, adenoviruses may possess some viral function similar to that of the polyoma large T antigen.

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LITERATURE CITED

1. **Bos, J. L., L. J. Polder, R. Bernards, P. I. Schrier, P. J. van den Elsen, A. J. van der Eb, and H. van Ormondt.** 1981. The 2.2 Kb E1B mRNA of human Ad 12 and Ad 5 codes for two tumor antigens starting at different AUG triplets. Cell **27**:121–131.
2. **Della Valle, G., R. G. Fenton, and C. Basilico.** 1981. Polyoma large T antigen regulates the integration of viral DNA sequences into the genome of transformed cells. Cell **23**:347–355.
3. **Dijkema, R., B. M. M. Dekker, M. J. M. van der Feltz, and A. J. van der Eb.** 1979. Transformation of primary rat kidney cells by DNA fragments of weakly oncogenic adenoviruses. J. Virol. **32**:943–950.
4. **Ezoe, H., R. B. Lai Fatt, and S. Mak.** 1981. Degradation of intracellular DNA in KB cells infected with *cyt* mutants of human adenovirus type 12. J. Virol. **40**:20–27.
5. **Ezoe, H., and S. Mak.** 1974. Comparative studies on functions of human adenovirus type 12 and its low oncogenic mutant virions. J. Virol. **14**:733–739.
6. **Frost, E., and J. Williams.** 1978. Mapping temperature sensitive and host range mutations of adenovirus type 5 by marker rescue. Virology **91**:39–50.
7. **Girvitz, S. C., S. Bacchetti, A. J. Rainbow, and F. L. Graham.** 1980. A rapid and efficient procedure for the purification of DNA from agarose gels. Anal. Biochem. **106**:492–496.
8. **Graham, F. L., P. J. Abrahams, C. Mulder, H. L. Heijneker, S. O. Warnaar, F. A. J. de Vries, W. Fiers, and A. J. van der Eb.** 1974. Studies on *in vitro* transformation by DNA and DNA fragments of human adenoviruses and simian virus 40. Cold Spring Harbor Symp. Quant. Biol. **39**:637–650.
9. **Graham, F. L., T. Harrison, and J. Williams.** 1978. Defective transforming capacity of adenovirus type 5 host-range mutants. Virology **86**:10–21.
10. **Graham, F. L., J. Smiley, W. C. Russell, and R. Nairn.** 1977. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J. Gen. Virol. **36**:59–72.
11. **Halbert, D. N., D. J. Spector, and H. J. Raskas.** 1979. In vitro translation products specified by the transforming region of adenovirus type 2. J. Virol. **31**:621–629.
12. **Harrison, T., F. Graham, and J. Williams.** 1978. Host range mutants of adenovirus type 5 defective for growth in HeLa cells. Virology **77**:319–329.
13. **Hassell, J. A., W. C. Topp, D. B. Rifkin, and P. E. Moreau.** 1980. Transformation of rat fibroblasts by cloned polyoma DNA fragments containing only part of the early region. Proc. Natl. Acad. Sci. U.S.A. **77**:3978–3982.
14. **Houweling, A., P. J. van den Elsen, and A. J. van der Eb.** 1980. Partial transformation of primary rat cells by the left most 4.5% fragment of adenovirus 5 DNA. Virology **105**:537–550.
15. **Jochemsen, H., G. S. G. Daniels, J. H. Lupker, and A. J. van der Eb.** 1980. Identification and mapping of the early gene products of adenovirus type 12. Virology **105**:551–563.
16. **Jones, N., and T. Shenk.** 1979. Isolation of adenovirus type 5 host range deletion mutants defective for transformation of rat embryo cells. Cell **17**:683–689.
17. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
18. **Lai Fatt, R. B., and S. Mak.** 1982. Mapping of an adenovirus function involved in the inhibition of DNA degradation. J. Virol. **42**:969–977.
19. **Laskey, R. A., and D. Mills.** 1975. Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. Eur. J. Biochem. **56**:335–341.
20. **Lassam, N. J., S. T. Bayley, and F. L. Graham.** 1978. Synthesis of DNA, late polypeptides, and infectious virus by host-range mutants of AD 5 in non-permissive cells. Virology **87**:463–467.
21. **Lassam, N. J., S. T. Bayley, and F. L. Graham.** 1979. Tumor antigens of human Ad 5 in transformed cells and in cells infected with transformation-defective host range mutants. Cell **18**:781–791.
22. **Mak, I., H. Ezoe, and S. Mak.** 1979. Structure and function of adenovirus type 12 defective virions. J. Virol. **32**:240–250.
23. **Mak, I., and S. Mak.** 1977. Synthesis and accumulation of polyadenylated and nonpolyadenylated virus-specific RNA in adenovirus-infected cells. Virology **82**:380–391.
24. **Mak, S., I. Mak, J. R. Smiley, and F. L. Graham.** 1979. Tumorigenicity and viral gene expression in rat cells transformed by Ad 12 virions or by the Eco RI C fragment of Ad 12 DNA. Virology **98**:456–460.
25. **McBride, W. D., and A. Wiener.** 1964. *In vitro* transformation of hamster kidney cells by human adenovirus type 12. Proc. Soc. Exp. Biol. Med. **115**:870–874.
26. **Moore, J. L., K. Chowdhury, M. A. Martin, and M. Israel.** 1980. Polyoma large tumor antigen is not required for tumorigenesis mediated by viral DNA. Proc. Natl. Acad. Sci. U.S.A. **77**:1336–1340.
27. **Ortin, J., K.-H. Scheidtmann, R. Greenberg, M. Westphal, and W. Doerfler.** 1976. Transcription of the genome of adenovirus type 12. III. Maps of stable RNA from productively infected human cells and abortively infected and transformed hamster cells. J. Virol. **20**:355–372.
28. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–252.
29. **Ross, S. R., S. J. Flint, A. J. Levine.** 1980. Identification of adenovirus early proteins and their genomic map positions. Virology **100**:419–432.
30. **Schauffhausen, B. S., J. Silver, and T. L. Benjamin.** 1978. Tumor antigen(s) in cells productively infected by wild-type polyoma virus and mutant NG-18. Proc. Natl. Acad. Sci. U.S.A. **75**:79–84.
31. **Sekikawa, K., K. Shiroki, S. Shimojo, S. Ojima, and K. Fujinaga.** 1978. Transformation of a rat cell line by an adenovirus 7 DNA fragment. Virology **88**:1–7.
32. **Shiroki, K., H. Handa, H. Shimojo, S. Yano, S. Ojima, and K. Fujinaga.** 1977. Establishment and characterization of

rat cell lines transformed by restriction endonuclease fragments of adenovirus 12 DNA. Virology **82**:462–471.

33. **Shiroki, K., K. Maruyama, I. Saito, Y. Fukui, and H. Shimojo.** 1981. Incomplete transformation of rat cells by a deletion mutant of adenovirus type 5. J. Virol. **38**:1048–1054.
34. **Shiroki, K., H. Shimojo, Y. Sawada, Y. Uemizu, and K. Fujinaga.** 1979. Incomplete transformation of rat cells by a small fragment of adenovirus 12 DNA. Virology **95**:127–136.
35. **Smiley, J. R., and S. Mak.** 1978. Transcription map for adenovirus type 12 DNA. J. Virol. **28**:227–239.
36. **Solnick, D., and M. A. Anderson.** 1982. Transformation-deficient adenovirus mutant defective in expression of region 1A but not region 1B. J. Virol. **42**:106–113.
37. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
38. **Takemori, N.** 1972. Genetic studies with tumorigenic adenoviruses. III. Recombination in adenovirus type 12. Virology **27**:157–167.
39. **Takemori, N., J. L. Riggs, and C. Aldrich.** 1968. Genetic studies with tumorigenic adenoviruses. I. Isolation of cytocidal (cyt) mutants of adenvirus type 12. Virology **35**:575–586.
40. **Takemori, N., J. L. Riggs, and C. Aldrich.** 1969. Genetic studies with tumorigenic adenoviruses. II. Heterogeneity of cyt mutants of adenovirus type 12. Virology **38**:8–15.
41. **Tibbetts, C.** 1977. Physical organization of subgroup B human adenovirus genomes. J. Virol. **24**:564–579.
42. **Wahl, G. M., M. Stern, and G. R. Stark.** 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl paper and rapid hybridization by using dextran sulfate. Proc. Natl. Acad. Sci. U.S.A. **76**:3683–3687.
43. **Weinstock, R., R. Sweet, M. Weiss, H. Cedar, and R. Axel.** 1978. Intragenic spacers interrupt the ovalbumin gene. Proc. Natl. Acad. Sci. U.S.A. **75**:1299–1303.