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In Vitro Transformation by the Adenovirus-Simian Virus 40 Hybrid Viruses

V. Virus-Specific Ribonucleic Acid in Cell Lines Transformed by the Adenovirus 2-Simian Virus 40 and Adenovirus 12-Simian Virus 40 Transcapsidant Hybrid Viruses

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The ribonucleic acid-deoxyribonucleic acid hybridization technique was utilized to determine the presence of adenovirus (ad) and SV40 genetic information and to determine which ad genomes were present in clones of hamster cells transformed with the ad 2-SV40 and ad 12-SV40 transcapsidant hybrid virus populations. The results were correlated with the morphology of the transformed cells and colonies. It was found that cells transformed by either transcapsidant virus which had an SV40 morphology contained the ad 7 and SV40 genomes, whereas cells with a typical ad morphology contained only ad genetic information. Cells and colonies with morphological features of both ad- and SV40-transformed cells contained either the ad 2, or ad 12 genomes, depending on the transcapsidant used, together with the ad 7 and SV40 genomes. The results indicate the following: at least three different events occurred during transformation of hamster cells by the transcapsidant virus populations; the morphology of the resulting clones is determined by the viral genome(s) present; the linkage of the ad 7-SV40 genomes is confirmed since the ad 7-SV40 genomes were never found to be dissociated; the defective ad 7-SV40 genomes are capable of causing transformation; and the transcapsidant particle is probably composed of only ad 7 and SV40 genetic information.

The preceding paper described the isolation and some properties of clones derived from hamster cell lines transformed by the adenovirus (ad) 2-SV40 (ad 2^{+t7}) and ad 12-SV40 (ad 12^{+t7}) transcapsidant hybrid virus populations (5). Three types of clones with different cellular morphologies were recognized. Although these data suggested that three different transformation events had occurred, it was difficult to determine which ad genome(s) were present in the clones. For example, since ad 2 and ad 7 tumor (T) antigens could not be demonstrated, it was not possible to ascertain whether ad 7 genetic information was present in ad 12^{+t7}-transformed cells, or whether ad 2^{+t7}-transformed cells contained either ad 2 or ad 7 genetic information, or both. That some ad determinants were present in many of the clones was indicated by the morphology of the transformed cells and of the tumors. However, since members of the three adenovirus subgroups produce morphologically similar transformed cells and tumors, the precise identification of the responsible ad genome(s) could not be made. Moreover, the absence of ad genetic information could not be inferred by the absence of typical ad morphology of the transformed cells or tumors. Consequently, a more direct approach was needed to delineate which viral genomes were present in the transformed cell lines. The technique of ribonucleic acid (RNA)-deoxyribonucleic acid (DNA) hybridization has previously been employed to demonstrate virus-specific RNA in cells transformed by members of each of the human ad subgroups (13-15), and in SV40- (1, 3, 19) and polyoma-transformed (3) cells; both SV40- and polyoma-specific RNA's were present in cells doubly transformed by these two viruses (3). Moreover, the virus-specific RNA from cells

transformed by each of the ad subgroups was found to be subgroup specific (13–15).

With this technique of RNA-DNA hybridization, we have investigated the presence of ad- and SV40-specific RNA's in clones derived from hamster cells transformed by the ad 2^{+t7} and 12^{+t7} transcapsidant hybrid viruses. The results were correlated with the morphological data presented in the previous paper (5).

MATERIALS AND METHODS

Cells. The origin and properties of the clones established from hamster cells transformed by the Ad 2^{+t7} and ad 12^{+t7} transcapsidant hybrid viruses are described in the preceding paper (5). Hamster cell lines originating from transformations by the ad 7-SV40 passage hybrid virus (ad 7^{+}HK-1 and ad 7^{+}HK-4) and by ad 12 (ad 12 HK-1) have also been described (10). The cells were grown in roller bottles (model no. 7013, Bellco Glass, Inc.) or 32-oz prescription bottles and were maintained in NCTC 109 medium which contained 10% fetal calf serum (FCS), 2 mM glutamine, penicillin (250 units/ml), and streptomycin (250 μg/ml). Parallel cultures of one cell line (ad 2^{+t7} HK-4) were maintained in medium containing 0.1 and 1.8 mM $CaCl_2$ (5). The bottles were rolled at 0.7 rev/min (Bellco cell production apparatus no. 7510) and were maintained at 37 C. The medium was changed at 3-day intervals until confluent monolayers were formed.

Radiolabeling of transformed cells. Roller bottle cultures were refed 1 day after reaching confluence; 18 to 22 hr later the medium was removed and uridine-5-^{3}H (22 to 30 c/mmole; Nuclear-Chicago Corp., Des Plaines, Ill.) was added to a final concentration of 25 μc/ml in a total of 35 ml. After 6 hr the bottles were drained, and the cells were trypsinized and washed as previously described (18). The washed cells were shipped in dry ice without loss of virus-specific RNA.

Radiolabeling of acutely infected cells. Confluent human embryonic kidney (HEK) monolayer cultures (roller bottles) were infected with a multiplicity of 10 to 50 $TCID_{50}$ per cell of ad 2, ad 7, or ad 12. The cells were labeled 16 to 20 hr later as described for the transformed cells, incubated at 37 C for 3 hr, and harvested by scraping.

Confluent monolayers (roller bottles) of the VERO line (a PPLO free line used at the 134th passage level) established from African green monkey kidney cells were infected with a multiplicity of 20 plaque forming units per cell of SV40 virus. The cells were labeled 32 hr later as described for the transformed cells, incubated at 37 C for 6 hr, and harvested.

Extraction of radioactively labeled RNA. The RNA extraction procedure was a modification (18) of the hot phenol-SDS (sodium dodecyl sulfate) method described by Scherrer and Darnell (23), and Kirby (17).

Viruses. Ad 2 (strain adenoid 6), ad 3 (GB strain), ad 7 (Goman strain), and ad 12 (Hull strain) were maintained by serial passage in either HEK or KB cells. The pools used for DNA extraction were purified by Paul Burnett of the Eli Lilly Co., Indianapolis, Ind. (11). SV40 strain 777 (8) was maintained by serial passage in BSC-1 cells and the virus was purified by the method of Black et al. (6).

Extraction of virus DNA. The DNA was extracted from the viruses by papain digestion of the virus particles and then by SDS-phenol extraction (20).

RNA-DNA hybridization. The technique of Gillespie and Spiegelman for hybridization of radioactive RNA with DNA trapped on a nitrocellulose filter (16) was modified as previously described (18). The reaction was performed in 250 μliters in 2 × SSC (0.15 M sodium chloride and 0.015 M sodium citrate, *p*H 6.7) + .05% SDS for 19 to 21 hr at 60 C. Nitrocellulose filters (47 mm HAWP, Millipore Filter Corp.) contained 0.75 to 1.0 μg of virus DNA per 13-mm filter. The single lot of filter paper used for these experiments retained more than 95% of the filtered DNA; less than 5% of the retained DNA was eluted during a 20-hr incubation. Only those values exceeding the *Escherichia coli* DNA blank by 100% were considered significant.

Determination of DNA content. Total DNA of the cultures listed in Table 1 was calculated from the DNA present in a 5% sample of those cultures. Nucleic acid extracts were prepared by the method of Schneider (24) and assayed for DNA content by the Burton modification of the diphenylamine reaction (12) with calf thymus DNA (Calbiochem, Los Angeles, Calif.) as a standard.

RESULTS

Conditions for detecting virus-specific RNA. The initial experiments were performed to determine optimal conditions for the detection of SV40-specific RNA. Extending the labeling time for roller cultures from 3 to 6 hr (Table 1) resulted in a proportionate increment of virus-specific RNA and increased the per cent of the total input ^{3}H-RNA that was virus-specific. Although the spinner culture had as much virus-specific RNA per cell (counts/min per μg of DNA) as the 6-hr roller culture, the virus-specific RNA represented a smaller per cent of the total input ^{3}H-RNA. Since the total input of radioactivity influences the counts/min retained by the blank (*E. coli*) filter, and since the spinner procedure was technically more difficult with no advantage, all succeeding experiments were performed with roller cultures labeled for 6 hr.

Specificity of hybridization reaction. The specificity of the hybridization technique is demonstrated in Table 2. Radioactive RNA from the untransformed hamster kidney cell line (BHK-21) did not react with any of the virus DNA's. Radioactive RNA from SV40-infected cells hybridized only with SV40 DNA and there was no hybridization between RNA's from ad-infected cells and SV40 DNA. There was minimal hybridization between the RNA's from cells in-

TABLE 1. *Effect of radioisotope labeling conditions on the production of virus-specific RNA in transformed cells*

Type of culture[a]	Hr of labeling	Total DNA	Total ^{3}H-RNA	Virus-specific ^{3}H-RNA[b]	Per cent hybridized[c]	Ratio of virus-specific counts/min to μg of DNA
		μg	*counts/min*	*counts/min*		
Roller	3	2,820	15.9×10^6	420	26×10^{-4}	0.15
Roller	6	2,540	18.2×10^6	935	51×10^{-4}	0.37
Spinner	6	1,970	26.9×10^6	690	25×10^{-4}	0.35

[a] Two confluent roller bottle cultures of an ad 2-SV40-transformed hamster cell line [ad 2^{++} HK-1 clone 3 P no. 8 (7)] were labeled as described in the text, and a third was trypsinized to make a spinner culture (5×10^5 cells/ml) before tritiated uridine was added.

[b] Net ^{3}H-RNA counts/min bound to a 1.0 μg SV40 DNA filter, determined in duplicate. Background (counts/min bound to 1.0-μg *E. coli* DNA filters; 2×10^{-4}% of input) has been subtracted.

[c] Ratio of total virus-specific ^{3}H-RNA (counts/min) to total ^{3}H-RNA input (counts/min).

TABLE 2. *Specificity of hybridization reaction*

Source of cellular RNA	Condition[a]	Input ^{3}H-RNA per vial (counts/min)	Counts/min of ^{3}H-RNA bound to virus DNA filter tested[b]					
			Ad 2	Ad 3	Ad 7	Ad 12	SV40	*E. coli*
BHK-21.....	None	10.0×10^5	0	0	0	0	2	2
VERO.....	SV40 infection	2.90×10^5	−2		0	1	1,112	4
HEK.....	Ad 2 infection	5.75×10^5	2,680		109	21	−1	5
HEK.....	Ad 7 infection	2.40×10^5	258	6,551	8,920	133	2	2
HEK.....	Ad 12 infection	6.50×10^5	44		23	4,950	5	2
Ad 12 HK-1..	Ad 12 transformed	1.25×10^5	7	9	8	397	6	8

[a] Virus infection and labeling of RNA as described in the text.

[b] Counts/min of ^{3}H-RNA bound to 1.0-μg (ad 2, ad 3, ad 12, SV40) or 0.75-μg (ad 7) filters; determination in duplicate; note that background (1.0-μg *E. coli* DNA filters) has not been subtracted.

fected by one of the adenovirus subgroups (as represented by ad 2, ad 7, and ad 12) and DNA from another subgroup. The reaction with heterologous ad DNA was always less than 5% of that which occurred with the homologous DNA. On the other hand, the expected sharing of nucleotide sequences within an adenovirus subgroup was demonstrated by the efficient hybridization of RNA from ad 7-infected cells with ad 3 DNA.

This specificity was maintained in transformed cell lines; ^{3}H-RNA from hamster kidney cells transformed by ad 12 (ad 12 HK-1) was specifically retained (in excess of the *E. coli* control) only by ad 12 DNA.

Virus-specific RNA in cell lines transformed by ad 7-SV40 passage hybrids. The specificity of the hybridization technique was further tested on cells transformed by ad 7-SV40 passage hybrid viruses (Table 3). ^{3}H-RNA specifically retained by SV40 DNA and ad 7 DNA was demonstrated. One-fourth as much RNA was bound to ad 3 as to ad 7 DNA. This finding and the negligible binding to ad 2 and ad 12 DNA are similar to results previously reported with ^{3}H-RNA from ad 7 tumor cells (14). It is readily apparent that the presence of ad 7 virus-specific RNA resulted in no cross-reaction with ad 2 or ad 12 DNA. The per cent of total input ^{3}H-RNA that was virus-specific (per cent hybridized) varied among these transformed cell lines and those to be discussed below, and even varied between passage levels of the same line. This may be due to differences in growth characteristics noted between the lines, and the unavoidable variation in cell density between confluent cultures. The specific activity of the ^{3}H-RNA varied over a threefold range (1×10^4 to 3×10^4 counts/min per μg of RNA).

Virus-specific RNA in cell lines transformed by ad 2^{+t7} or ad 12^{+t7} transcapsidant hybrid viruses. The mass cultures of both cell lines transformed by the ad 2^{+t7} transcapsidant hybrid virus contained ad 2-, ad 7-, and SV40-specific RNA molecules (Table 4). The ad 2^{+t7} HK-3 cell line was cloned and two types of clones were obtained: one type similar in nucleic acid content to the parent cell line (clones 8, 10, and 12), and a second

TABLE 3. *Virus-specific RNA in cells transformed by Ad 7-SV40 passage hybrid viruses*

Line	Input of ^{3}H-RNA per vial (counts/min)	Per cent hybridized[a] on virus DNA filter tested				
		Ad 2	Ad 3	Ad 7	Ad 12	SV40
Ad 7$^+$ HK1	0.83×10^6	0.1×10^{-4}	13×10^{-4}	58×10^{-4}	0	86×10^{-4}
Ad 7$^+$ HK4	1.88×10^6	0.4×10^{-4}	4.3×10^{-4}	15.4×10^{-4}	0	40×10^{-4}

[a] Virus-specific ^{3}H-RNA (counts/min)/^{3}H-RNA input (counts/min); virus-specific ^{3}H-RNA retained on a 1.0-μg (ad 2, ad 12, SV40) or 0.75-μg (ad 7) DNA filter was determined in duplicate; background counts/min bound to 1.0-μg *E. coli* DNA filters; $< 2 \times 10^{-4}$% of input) has been subtracted.

TABLE 4. *Virus-specific RNA's in cells transformed by the ad 2^{+t7} transcapsidant hybrid virus*

Line	Input ^{3}H-RNA per vial (counts/min)	Per cent hybridized[a] on virus DNA filter tested			
		Ad 2	Ad 7	Ad 12	SV40
Ad 2^{+t7} HK3					
(Mass)	2.3×10^6	64×10^{-4}	195×10^{-4}	0	240×10^{-4}
Clone 1					
Clone 3	5.2×10^6	0	12×10^{-4}	0	32×10^{-4}
Clone 8	6.4×10^6	10×10^{-4}	27×10^{-4}	0	54×10^{-4}
Clone 10	4.0×10^6	14×10^{-4}	130×10^{-4}	0	251×10^{-4}
Clone 12	3.1×10^6	45×10^{-4}	13×10^{-4}	0	56×10^{-4}
Clone 15	6.95×10^6	0	12.4×10^{-4}	0	72×10^{-4}
Ad 2^{+t7} HK4					
(Mass)	1.8×10^6	4×10^{-4}	90×10^{-4}	1×10^{-4}	191×10^{-4}
(Mass-low Ca^{++})[b]	3.8×10^6	5×10^{-4}	23×10^{-4}	0	166×10^{-4}
(Mass-normal Ca^{++})[b]	4.4×10^6	9×10^{-4}	13×10^{-4}		71×10^{-4}
Clone 1	4.2×10^6	9×10^{-4}	30×10^{-4}	0	76×10^{-4}
Clone 3	4.7×10^6	15×10^{-4}	17×10^{-4}	0	162×10^{-4}
Clone 6	4.7×10^6	6×10^{-4}	21×10^{-4}	0	41×10^{-4}
Clone 7	4.5×10^6	23×10^{-4}	30×10^{-4}	0	118×10^{-4}
Clone 8	4.4×10^6	7×10^{-4}	19×10^{-4}	0	59×10^{-4}

[a] Virus-specific ^{3}H-RNA (counts/min)/^{3}H-RNA input (counts/min); virus-specific ^{3}H-RNA retained on a 1.0-μg (ad 2, ad 12, SV40) or 0.75-μg (ad 7) DNA filter was determined in duplicate; background (counts/min bound to 1.0-μg *E. coli* DNA filters; $<2 \times 10^{-4}$% of input) has been subtracted.

[b] Low Ca^{++} media contained 0.1 mM calcium; normal Ca^{++} media contained 1.8 mM calcium.

type which contained ad 7- and SV40-specific RNA forms but no ad 2-specific RNA (clones 3 and 15). All clones tested from the HK-4 line were similar to the parent line, and the calcium concentration of the medium did not alter the type of virus-specific RNA obtained.

Clones from five cell lines transformed by the ad 12^{+t7} transcapsidant hybrid virus population were examined (Table 5). Three separate cell populations were isolated. Clones from the HK-2 cell line contained only ad 7- and SV40-specific RNA's. The mass culture of the HK-3 cell line gave rise to two types of clones: those containing Ad 7- and SV40-specific RNA's (clones 2 and 5), and those containing ad 12-specific RNA (clones 10 and 12). The mass culture and clones of the HK-1 and HK-4 cell lines contained ad 7-, ad 12-, and SV40-specific RNA's. Two clones from the HK-5 cell line contained only ad 7- and SV40-specific RNA's (clones 1 and 6), whereas clone 10 contained ad 7, ad 12, and SV40-specific RNA's.

The detection of ad 7-specific RNA in those cell lines containing SV40-specific RNA is consistent with the strong evidence for linkage between these two viral genomes in the ad 7-SV40 hybrid virus (2, 22). However, in those instances where smaller amounts of another ad-specific RNA was demonstrated (e.g., ad 2-specific RNA in certain ad 2^{+t7} clones) it could be argued that this merely represented a small nucleotide sequence shared between the ad subgroups. Although the specificity studies (Table 2) indi-

TABLE 5. *Virus-specific RNA's in cells transformed by the ad 12^{+t7} transcapsidant hybrid virus*

Line	Input ^{3}H-RNA per vial (counts/min)	Per cent hybridized[a] on virus DNA filter tested			
		Ad 2	Ad 7	Ad 12	SV40
Ad 12^{+t7} HK1					
Clone 1	2.5×10^6	1.4×10^{-4}	41×10^{-4}	54×10^{-4}	133×10^{-4}
Clone 3	5.3×10^6	1.9×10^{-4}	16	40	39×10^{-4}
Ad 12^{+t7} HK2					
(Mass)	2.1×10^6	0.9×10^{-4}	10×10^{-4}	0.2×10^{-4}	18×10^{-4}
Clone 3	3.4×10^6	0	4.9×10^{-4}	0	20×10^{-4}
Clone 4	4.0×10^6	0.5×10^{-4}	4.0×10^{-4}	0	27×10^{-4}
Ad 12^{+t7} HK3					
(Mass)	1.9×10^6	0	2×10^{-4}	105×10^{-4}	3×10^{-4}
Clone 2	5.2×10^6	0	16×10^{-4}	0	36×10^{-4}
Clone 5	4.8×10^6	0.4×10^{-4}	15×10^{-4}	0	90×10^{-4}
Clone 10	3.8×10^6	0.9×10^{-4}	0	20×10^{-4}	0
Clone 12	3.5×10^6	0	0.4×10^{-4}	41×10^{-4}	0
Ad 12^{+t7} HK4					
(Mass)	4.6×10^6	0.5×10^{-4}	33×10^{-4}	31×10^{-4}	60×10^{-4}
Clone 1	3.9×10^6	0	11×10^{-4}	63.5×10^{-5}	62×10^{-4}
Clone 3	3.7×10^6	0	24×10^{-4}	26×10^{-4}	33×10^{-4}
Ad 12^{+t7} HK5					
Clone 1	2.5×10^6	1.1×10^{-4}	92×10^{-4}	0.2×10^{-4}	230×10^{-4}
Clone 6	2.0×10^6	0.5×10^{-4}	23×10^{-4}	0.1×10^{-4}	40×10^{-4}
Clone 10	2.5×10^6	0	13×10^{-4}	10×10^{-4}	69×10^{-4}

[a] Virus-specific ^{3}H-RNA (counts/min)/^{3}H-RNA input (counts/min); virus-specific ^{3}H-RNA retained on a 1.0-μg (ad.2, ad.12, SV40) or 0.75-μg (ad 7) DNA filter was determined in duplicate; background (counts/min bound to 1.0-μg *E. coli* DNA filters; $<2 \times 10^{-4}$% of input) has been subtracted.

cate that such shared sequences are rare, if this type of unique area of the ad 7 genome were transcribed repetitively many times, we would observe considerable hybridization with an heterologous ad DNA.

Thus, a hybridization-competition experiment was performed to demonstrate that the ad 2-specific RNA shared no nucleotide sequence with the ad 7-specific RNA in the ad 2^{+t7} HK-4 (clone 7) cell line (Table 6). Unlabeled RNA was prepared from ad 2- and ad 7-infected HEK cells. The unlabeled ad 7-specific RNA saturated the available sites on ad 7-DNA filters and prevented hybridization by ad 7-specific radioactive RNA. Unlabeled ad 2 RNA was an equally effective competitor in the reaction between ad 2 DNA and radioactive ad 2 RNA. Equivalent amounts (in terms of ability to compete against homologous RNA) of unlabeled ad 2-specific and ad 7-specific RNA's were then tested for their ability to compete in the hybridization reaction between radioactive ad 2^{+t7} RNA and ad 2-DNA filters. It can be seen that ad 7-specific RNA failed to compete in the hybridization reaction and that ad 2-specific RNA was an efficient competitor. Thus, this cell line contains nucleotide sequences unique to ad 2 DNA that are not shared with the ad 7 genome.

DISCUSSION

Optimal conditions were obtained for the detection of virus-specific RNA's in hamster cells transformed by the ad-SV40 transcapsidant hybrid viruses. The specificity of the RNA-DNA hybridization technique permitted the simultaneous detection of parts of two different adenovirus genomes and also SV40 genetic information in some cell clones. Every transformed clone which contained SV40-specific RNA also contained ad 7-specific RNA. This finding confirms previous studies in which the linkage of SV40 and ad 7 DNA molecules had been demonstrated by antigenic (22) and biophysical techniques (2). Thus the ad 7-SV40 genomes, transferred to a new adenovirion during the process of transcapsidation, remain linked while integrated in the transformed cell; additional evidence for this

TABLE 6. *Competition of unlabeled ad 2- and ad 7-specific RNA in the ad 2^{+t7} hybridization reaction*

Radioactive RNA[a]	Virus DNA tested[b]	Competitor RNA	Competitor RNA input (μg)	Counts/min of ^{3}H-RNA bound to filter[c]	Per cent of competition[d]
Ad. 7	Ad 7	0	0	911	0
	Ad 7	Ad 7	160	120	84
Ad. 2	Ad 2	0	0	140	0
	Ad 2	Ad 2	200	7	95
Ad 2^{+t7} HK4 Clone 7	Ad 2	0	0	88	0
	Ad 2	Ad 7	120	88	0
	Ad 2	Ad 2	150	15	83
	Ad 2	WHK[e]	250	91	0

[a] Radioactive RNA was prepared from ad 2- or ad 7-infected HEK cells and from the ad 2^{+t7} HK4 clone 7-transformed cell line. Specific activity of the RNA was approximately 50,000 counts/min per μg of RNA for the ad 2 and ad 7-specific RNA and 25,000 counts/min for the ad 2^{+t7} RNA. Paired rollers were prepared unlabeled to use for competitor RNA.

[b] Ad 7-DNA filters (0.038 μg) and ad 2 DNA filters (0.058 μg) were used in the ad 7 vs. ad 7, and ad 2 vs. ad 2 competition experiments; ad 2 DNA filters (0.258 μg) were used in the ad 2^{+t7} competition experiments.

[c] Duplicate determinations with background (counts/min bound to 0.038- or 0.25-μg *E. coli* DNA filters; $<10^{-4}$% of input) was subtracted.

[d] Values determined by [1-(counts/min of ^{3}H-RNA bound in presence of competitor)/(counts/min of ^{3}H-RNA bound in absence of competitor)] $\times$ 100.

[e] Weanling hamster kidney.

linkage is its persistence throughout all cloning procedures.

The nucleic acid hybridization technique has indicated the presence of viral genetic material directly and more precisely than other methods now available. Thus the presence of ad 2 and ad 7 DNA's has been established by the presence of ad 2- and ad 7-specific RNA when the presence of these genomes could not be demonstrated by T antigen studies (5). The nucleic acid hybridization data reported here are correlated in Table 7 with the morphological description of the clones presented in the preceding paper (5).

It is postulated that at least three different transformation events occurred in hamster kidney cells transformed by the ad 2^{+t7} and ad 12^{+t7} transcapsidant virus populations. Clones representing these events could be isolated from the mass cultures, and the discussion will be limited to these clones. Two clones (ad 12^{+t7} HK3, clones 10 and 12) had a typical adenovirus morphology and contained only ad 12 T antigen and ad 12-specific RNA. These clones were presumably derived from cells transformed by the ad 12 virions in the transcapsidant population.

The remaining clones contained both adenovirus and SV40 genetic information. Those clones having an SV40 type morphology (ad 2^{+t7} HK3, clones 1, 3, and 15; ad 12^{+t7} HK2, clones 3 and 4; ad 12^{+t7} HK3, clones 2 and 5; ad 12^{+t7} HK5, clones 1 and 6) contained only ad 7- and SV40-specific RNA molecules. This suggests that the transcapsidant particle responsible for transformation was composed of only ad 7 and SV40 DNA molecules enclosed in an ad 2 or ad 12 coat protein. Thus the process of transcapsidation may consist of the coating of the defective ad 7-SV40 genome with the coat protein of another adenovirus. This would be compatible with the rapidity with which transcapsidation occurs (21). That the defective ad 7-SV40 genome can cause transformation is revealed by dose response curves of transformation with the ad 7-SV40 hybrid population (4). These studies revealed that transformation of hamster kidney cells occurred with one-hit kinetics, and that all the transformed cell lines derived contained the SV40 T antigen. The ad 7 virus derived from the hybrid population does not transform hamster cells (9). It is, therefore, probable that the transformation event occurred as a result of infection with the ad 7-SV40 hybrid particle, and that the hybrid particle, although defective with respect to infectivity, is capable of causing transformation.

The remaining clones had morphological features of both SV40- and ad-transformed cells (Table 7) and contained ad 2- or ad 12-specific RNA, depending on the transcapsidant virus used, together with the ad 7-SV40 genome. Therefore it is apparent that SV40 determinants predominate when the SV40 genome is covalently linked to the ad 7 genome; however, with the addition of ad 2 or ad 12 genetic information

TABLE 7. *Summary of the morphological characteristics and virus-specific RNA's in ad 2^{+t7}- and ad 12^{+t7}-transformed cell clones*

Cell line	Morphology[a]			Virus-specific RNA[b]			
	SV40	Ad	Intermediate	Ad 2	Ad 7	Ad 12	SV40
Ad 2^{+t7} HK-3	+		+	+	+		+
Clone 1	+						
Clone 3	+				+		+
Clone 8			+	+	+		+
Clone 10			+	+	+		+
Clone 12			+	+	+		+
Clone 15	+				+		+
Ad 2^{+t7} HK-4			+	+	+		+
Clone 1			+	+	+		+
Clone 3			+	+	+		+
Clone 6			+	+	+		+
Clone 7			+	+	+		+
Clone 8			+	+	+		+
Ad 12^{+t7} HK-1			+				
Clone 1			+		+	+	+
Clone 3			+		+	+	+
Ad 12^{+t7} HK-2	+				+		+
Clone 3	+				+		+
Clone 4	+				+		+
Ad 12^{+t7} HK-3	+	+			+	+	+
Clone 2	+				+		+
Clone 5	+				+		+
Clone 10		+				+	
Clone 12		+				+	
Ad 12^{+t7} HK-4			+		+	+	+
Clone 1			+		+	+	+
Clone 3			+		+	+	+
Ad 12^{+t7} HK-5	+		+				
Clone 1	+				+		+
Clone 6	+				+		+
Clone 10			+		+	+	+

[a] *See* Table 2 of the preceding paper (5).
[b] *See* Table 4 and Table 5.

adenovirus determinants are expressed as well, resulting in a cell of intermediate morphology. If the foregoing conclusions about the nature of the transcapsidant particle are correct, then it is likely that these clones arose from the dual infection of cells by the transcapsidant particle as well as the ad 2 or ad 12 virions present in the hybrid population. In all instances where the viral RNA determinations have been carried out with once and twice-cloned cells, they have been found to be identical, indicating that three viral genomes form a stable, heritable association with these cell clones.

In reconstruction experiments, coinfection with high multiplicities of both SV40 and ad 12 resulted in transformed cell lines which contained both SV40 and ad 12 T antigens in each cell and had an intermediate cell morphology (10). In the transformations carried out with the ad 12^{+t7} transcapsidant population, the multiplicity of infection (virus to cell) for both adenovirions and transcapsidant particles was 30 to 60, high enough to insure that the vast majority of cells were likely to be infected by both the transcapsidant particle as well as the complete adenovirions present, and nearly all the transformed foci which resulted were of an intermediate morphology (10). In the transformation experiments with the ad 2^{+t7} hybrid, the virus to cell multiplicity of infection was 0.3 to 3.0; it is quite

likely, therefore, that infection of cells would occur from a single particle and that most transformations occurred as a result of infection with the transcapsidant particle since all transformed cells contained the SV40 T antigen, and ad 2 virions alone failed to transform. This is consistent with the observation that the vast majority of transformations had an SV40-type morphology (9, 10).

Other explanations for the presence of three genomes in cells with intermediate morphology may be given. It is possible that the transcapsidant particle contains, in addition to the ad 7-SV40 genome, ad 12 or ad 2 DNA. These particles could represent true recombinants or could be particles containing unlinked ad 7-SV40 DNA, and ad 2 or ad 12 DNA of varying size. The transcapsidant hybrid populations utilized in these experiments were passed enough times (three to six passages after formation) to allow recombination to occur (21). A definitive description of the transcapsidant particle, however, will require its isolation in pure form and determination of its structure by biophysical or nucleic acid hybridization studies, or both.

Cells containing all three genomes could conceivably have arisen by somatic hybridization of cells transformed by pure adenovirions and the ad 7-SV40 transcapsidant particle. This is unlikely since no giant nuclei were seen and chromosomal analyses of one line containing all three genomes (ad 12^{+t7} HK-1) revealed no tetraploid mitotic figures (10).

In all the cell lines, the ratio of SV40-specific RNA to ad 7-specific RNA is greater than 1.0 and, in most instances, greater than 2.0. This does not, however, indicate the relative amounts of the virus genomes in the transformed cells, since the hybridization reaction yields information only about the quantity of virus-specific RNA present and not the nucleotide sequences present. Similarly, conclusions cannot be drawn from the ad 7 RNA to ad 2 RNA or the ad 7 RNA to ad 12 RNA ratios.

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