Transforming Region of Group A, B, and C Adenoviruses: DNA Homology Studies with Twenty-Nine Human Adenovirus Serotypes

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The 31 human adenovirus (Ad) serotypes form five groups based upon DNA genome homologies: group A (Ad12, 18, 31), group B (Ad3, 7, 11, 14, 16, 21), group C (Ad1, 2, 5, 6), group D (Ad8, 9, 10, 13, 15, 17, 19, 20, 22-30), and group E (Ad4) (M. Green, J. Mackey, W. Wold, and P. Rigden, *Virology*, in press). Group A Ads are highly oncogenic in newborn hamsters, group B Ads are weakly oncogenic, and other Ads are nononcogenic. However, most or all Ads transform cultured cells. We have studied the homology of Ad5, Ad7, and Ad12 transforming restriction endonuclease DNA fragments with DNAs of 29 Ad types. Ad5 *HindIII*-G (map position 0-7.3), Ad7 *XhoI*-C (map position 0-10.8), and Ad12 (strain Huie) *EcoRI*-C (map position 0-16) and *SalI*-C (map position 0-10.6) fragments were purified, labeled in vitro (nick translation), and annealed with DNAs of Ad1 to Ad16, Ad18 to Ad24, and Ad26 to Ad31. Hybrids were assayed by using hydroxylapatite. Ad5 *HindIII*-G hybridized 98 to 100% with DNAs of group C Ads, but only 1 to 15% with DNAs of other types. Ad7 *XhoI*-C fragment hybridized 85 to 99% with DNAs of group B Ads, but only 6 to 21% with DNAs of other types. Ad12 (Huie) *EcoRI*-C hybridized 53 to 68% with DNAs of five other Ad12 strains, 53% with Ad18 DNA, 56% with Ad31 DNA, but only 3 to 13% with DNAs of other types. In vitro-labeled Ad12 (Huie) *SalI*-C hybridized 35 to 71% with DNAs of 6 other Ad12 strains, 44% with Ad18 DNA, 52% with Ad31 DNA, but only 2 to 7% with DNAs Ad7, Ad2, Ad26, or Ad4. When assayed using S-1 nuclease, *SalI*-C annealed 17 to 44% with DNAs of group A Ads. The melting temperatures of the hybrids of Ad5 *HindIII*-G with all group C Ad DNAs were 84°C in 0.12 M sodium phosphate (pH 6.8). The melting temperature of the Ad12 (Huie) *EcoRI*-C hybrid with Ad12 (Huie) DNA was 83°C, but was only 71 to 77°C with DNAs of other group A Ads. Thus, group C and group B Ads both have very homologous transforming regions that are not represented in DNAs of non-group C Ads or non-group B Ads, respectively. Similarly, group A Ads have unique but less homologous transforming regions. These different transforming nucleotide sequences may be reflected in the different oncogenic properties of group A, B, and C Ads.

Since their discovery in 1953, at least 31, and perhaps as many as 35 (1, 23, 49) human adenovirus (Ad) serotypes (Ad1-35) have been identified (14, 42, 55). In 1962, Ad12 was reported to induce tumors when inoculated into newborn hamsters (26, 51). Similar inoculation studies soon showed that the Ad types differed in tumorigenicity: Ad12, Ad18 (26, 51), and Ad31 (41) produced tumors in most animals within 2 months, Ad3 (25), Ad7 (12, 28), Ad14, Ad16, and Ad21 (14, 15) produced tumors in a few animals within 4 to 18 months, and Ad1, Ad2, Ad4 (2), and Ad5 did not produce tumors. Using highly purified virus preparations, Green (15) confirmed the above results and showed that Ad14,

Ad16, and Ad21 were also tumorigenic, but was unable to induce tumors with many of the other types. The Ad-induced tumors were generally sarcomas or fibrosarcomas that did not metastasize. The tumors were transplantable and formed permanent cultured cell lines that were tumorigenic in hamsters. Ad12 was also shown to transform cultured hamster cells (35, 44). Sera from tumored hamsters reacted in complement fixation and fluorescent antibody tests with Ad-induced tumors, tumor cell lines, in vitro-transformed cells, and with "early" infected cells. The antigen(s) responsible for this reaction was called a T (tumor)-antigen. Based upon these tumorigenic properties and upon the immuno-

logical cross-reactions of tumor sera with tumor cells and infected cells, Huebner (24) proposed that the Ads be grouped into "highly oncogenic" group A (Ad12, 18, 31) and "moderately oncogenic" group B (Ad3, 7, 11, 14, 16, 21). This arrangement agreed quite well with the Ad hemagglutination properties (45) and the guanine plus cytosine content of the Ad duplex DNA genome (43).

In 1967, it was reported that Ad2 could transform cultured rat embryo cells (6). Ad1 (33), Ad5, and Ad6 (10) were also shown to transform cells. Using hamster sera directed against extracts of Ad1-SV40 and Ad2-SV40 induced hamster tumors, Gilden et al. (10) found that Ad1, Ad2, Ad5, and Ad6 shared a common T-antigen and thus represented a third group of Ads, group C. In contrast to cells transformed by group A and B Ads, most group C Ad-transformed rat cells do not induce tumors when inoculated into syngeneic rats, or even into nude mice (7). Group C Ad-transformed hamster cells, however, readily produce tumors (30, 53).

In 1969, McAllister and colleagues (34) reported that Ad9, Ad10, Ad15, Ad17, Ad19, and Ad26 were able to transform cultured rodent cells. Ad19- and Ad26-transformed NIL-2 hamster cells formed tumors when inoculated into hamsters. Based on reactivity of the hamster tumor sera, they proposed a fourth group of human Ads, group D (Ad8-10, 13, 15, 17, 19, 20, 22-30). Although McAllister et al. (34) did not detect viral DNA or RNA in the transformed cells, we have found that an Ad26-transformed cell line contains multiple copies of most of the Ad26 genome (unpublished data). Recently, Jonsson and Ankerst (27) reported that Ad9 induced mammary fibroadenomas in newborn female rats.

The above studies indicate that although only group A, and to a lesser extent group B, Ads are tumorigenic in newborn hamsters, most or all Ads can transform cultured rodent fibroblasts. The reason for this difference in oncogenic properties is not known. The transforming regions of group A, B, and C Ads have been mapped at the extreme left end of the viral genome. For example, cultured rat cells have been transformed by transfection with Ad5 or Ad2 *HindIII*-G fragment (map position 0-7.3) (13, 52), with Ad12 *EcoRI*-C (map position 0-16) (57; S. Mak, personal communication) and *HindIII*-G (map position 0-7.2) fragments, and with Ad7 *HindIII*-I-J fragment (map position 0-8.1) (47). All Ad-transformed cells tested contain these transforming fragment sequences, plus variable portions of the remainder of the viral genome (3, 5, 8, 20, 21, 40). An early gene block (early genes

are expressed before initiation of viral DNA replication) has been mapped within approximate positions 1-11 on the group C (4, 5) and group A (40) Ad genomes. Thus, a subset of early genes (but no late genes) are expressed in cells transformed by group A, B, and C Ads (16). Presumably, a protein(s) encoded by these "transforming gene(s)" functions to maintain cell transformation. This protein(s) is a component of the T-antigen(s) present in Ad tumors and transformed cells discussed above.

An understanding of the genome relationships among the Ad groups may provide a molecular basis of the different in oncogenic properties and may illuminate mechanisms of Ad cell transformation. In this report we describe DNA-DNA homology studies on early gene block 1 (the transforming region) of 29 Ad serotypes, by using in vitro-labeled Ad5 *HindIII*-G, Ad7 *XhoI*-C (map position 0-10.8), and Ad12 *EcoRI*-C (map position 0-16) and *SalI*-C (map position 0-10.6) fragments as probes. In a separate study (M. Green, J. Mackey, W. Wold, and P. Rigden, Virology, in press), we have shown that 31 Ad serotypes can be arranged into five DNA homology groups, based upon molecular hybridization reactions with in vitro-labeled viral DNAs. The groups are as follows: group A (Ad12, 18, 31), group B (Ad3, 7, 11, 14, 16, 21), group C (Ad1, 2, 5, 6), group D (Ad8-10, 13, 15, 17, 19, 20, 22-30), and group E (Ad4).

MATERIALS AND METHODS

Cells and virus. The serotype of each Ad studied was verified by neutralization tests performed with standard reference sera. Ads were grown in KB cell suspension cultures; virions were purified by banding in CsCl gradients (17) and plaque assayed by using KB cell monolayers (19).

Preparation of Ad5 *HindIII*-G fragment, Ad7 *XhoI*-C fragment, and Ad12 (Huie) *EcoRI*-C and *SalI*-C fragments. Ad DNA was extracted by using papain as previously described (18, 56). *EcoRI* was purified by the method of Mulder and Delius (38). *HindIII* and *SalI*-C were purchased from New England Biolabs. Viral DNA was digested with restriction endonucleases; fragments were resolved by electrophoresis through agarose slab gels and eluted and purified as described earlier (20, 56).

In vitro labeling of restriction DNA fragments. Fragments were labeled in vitro by the "nick translation" reaction as described elsewhere (37, 56). Probes prepared by this method are uniformly labeled and hybridize well (37).

Hybridization conditions. All hybridizations were done for 4 h at 68°C in duplicate 50- μ l portions containing 4 μ g of test DNA per ml, 500 cpm of probe DNA, 0.72 M NaCl, 10 mM piperazine-*N,N'*-bis(2-ethanesulfonic acid) (pH 6.7), 1 mM EDTA, and 0.05% sodium dodecyl sulfate. Hybrids were assayed by

batchwise hydroxylapatite chromatography (20, 56). Some hybrids (Table 4) were assayed with the S-1 nuclease procedure (54).

Melting temperature determinations. Hybrids were loaded onto water-jacketed columns (60°C) containing 1 ml of hydroxylapatite equilibrated in 0.12 M sodium phosphate buffer (pH 6.8) and 0.4% sodium dodecyl sulfate, and the columns were washed with 15 to 20 ml of equilibration buffer. The temperature was increased in increments of 5°C up to 80°C and then in 2°C increments to 90 to 94°C, allowing 30 min for the column to equilibrate at each temperature. At each temperature 10 ml of equilibration buffer was passed through the column and collected, and the radioactivity was determined by liquid scintillation counting.

RESULTS

Preparation, in vitro labeling, and reassociation properties of Ad5 *Hind*III-G and Ad12 *Eco*RI-C and *Sal*I-C transforming DNA fragments. In initial studies the Ad12 *Eco*RI-C (map position 0-16) and Ad5 *Hind*III-G (map position 0-7.3) fragments were used to prepare group A and group C Ad-transforming fragment probes. In later studies, we also used the Ad12 *Sal*I-C (map position 0-10.6) fragment. Figure 1 shows the cleavage pattern of Ad5 DNA with *Hind*III (lane A) and of Ad12 (Huie) DNA with *Eco*RI (lane B) and *Sal*I (lane C). Similar cleavage patterns have been reported previously for Ad5 DNA with *Hind*III (52) and for Ad12 DNA with *Eco*RI (20, 38, 40) and *Sal*I (22, with correction in Cell 11:985, 1977). The Ad5 *Hind*III-G, Ad12 *Eco*RI-C, and Ad12 *Sal*I-C fragments were purified and labeled in vitro as described above. The reassociation properties of the three probes are shown in Fig. 2. The $C_{0t_{1/2}}$ of *Eco*RI-C was 8.0×10^{-4} , that of *Sal*I-C was 5.1×10^{-4} , and that of *Hind*III-G was 3.7×10^{-4} , consistent with the complexity of each fragment. At least 90% of each probe reassociated into duplex DNA. These probes were used in the transforming-region homology studies described below.

The Ad7 *Xho*I-C fragment was prepared, labeled, and characterized in a similar manner (data not shown). The *Xho*I cleavage pattern for Ad7 DNA appeared to be similar to that published by Tibbetts (50).

Homology of Ad5 *Hind*III-G DNA fragment with DNAs of 29 human Ad serotypes. In vitro-labeled Ad5 *Hind*III-G DNA fragment was hybridized with DNAs of 29 human Ad types (serotypes 17, 25, and presumptive 32 to 35 were not included in our studies). The results are summarized in Table 1. The serotypes are arranged into groups A through E. The *Hind*III-G probe hybridize 98 to 100% with Ad1, 2, 5, and 6, but only 1 to 15% with DNAs of the other serotypes. Similar results were obtained by using

FIG. 1. Cleavage patterns of Ad5 DNA with *Hind*III and of Ad12 (Huie) DNA with *Eco*RI and *Sal*I. Viral DNAs were cleaved with restriction endonucleases; fragments were resolved by electrophoresis through agarose gels, stained with ethidium bromide, and photographed under UV light. (A) Ad5 DNA with *Hind*III. (B) Ad12 DNA with *Eco*RI. (C) Ad12 DNA with *Sal*I.

FIG. 2. Reassociation of in vitro-labeled Ad5 *Hind*III-G, Ad12 *Eco*RI and Ad12 *Sal*I-C transforming DNA fragments. Probe DNAs were allowed to reassociate in the presence of differing amounts of unlabeled Ad5 (for *Hind*III-G) or Ad12 (for *Eco*RI-C and *Sal*I-C) DNAs. Duplicate aliquots were taken at appropriate times, and hybrids were assayed by using hydroxylapatite. C_0t refers to the product of concentration of nucleotides in moles of nucleotides per liter and the hybridization time in seconds, corrected to an Na^+ concentration of 0.18 M.

TABLE 1. Hybridization of *in vitro* ^3H -labeled Ad5 *Hind*III-G fragment with DNAs of 29 human Ad serotypes

DNA ^a	% Hybridization ^b
Group C	
Ad5	100.0
Ad1	98.4
Ad2	98.6
Ad6	98.9
Group B	
Ad3	4.1
Ad7	2.3
Ad11	2.1
Ad14	3.4
Ad16	4.0
Ad21	4.2
Group A	
Ad12 (Huie)	1.4
Ad18	0.6
Ad31	0.9
Group D	
Ad8	4.6
Ad9	8.1
Ad10	5.6
Ad13	3.3
Ad15	8.6
Ad19	4.7
Ad20	7.2
Ad22	3.4
Ad23	11.4
Ad24	6.9
Ad26	10.7
Ad27	7.8
Ad28	8.9
Ad29	8.2
Ad30	5.7
Group E	
Ad4	13.4
Other viruses	
SA-7	-0.2
SV40 ^c	3.7

^a The Ad serotype DNAs are presented as five groups, A through E, based upon entire DNA genome homologies (Green et al., Virology, in press). Ad17, 25, and 32 to 35 were not included in this study.

^b The self-annealing background of the probe (11.2% in the presence of 4 μg of KB cell DNA per ml) has been subtracted and the values have been normalized to maximum hybridization (87.8%) to Ad5 DNA.

^c SV40, Simian virus 40.

the *I*-strand of Ad2 *Hind*III-G fragment prepared from DNA labeled *in vivo* with [^3H]thymidine (data not shown). It is clear that Ad1, 2, 5, and 6 form a group (group C) with very homologous transforming region sequences and that at least 85 to 95% of these sequences are not represented in the DNAs of other serotypes.

Homology of Ad7 *Xho*I-C fragment with DNAs of 29 human Ad serotypes. Table 2 summarizes the hybridization results of *in vitro* ^{32}P -labeled Ad7 *Xho*I-C fragment with DNAs of

TABLE 2. Hybridization of *in vitro* ^{32}P -labeled Ad7 *Xho*I-C fragment with DNAs of 29 human Ad serotypes

DNA	% Hybridization ^a
Group B	
Ad3	98.7
Ad7	100.0
Ad11	85.1
Ad14	87.3
Ad16	99.0
Ad21	94.3
Group A	
Ad12 (Huie)	5.6
Ad18	10.8
Ad31	11.1
Group C	
Ad1	13.0
Ad2	10.1
Ad5	11.8
Ad6	12.9
Group D	
Ad8	17.1
Ad9	16.4
Ad10	13.8
Ad13	11.2
Ad15	20.4
Ad19	14.4
Ad20	19.3
Ad22	17.4
Ad23	14.9
Ad24	12.8
Ad26	14.1
Ad27	18.4
Ad28	17.2
Ad29	19.5
Ad30	17.7
Group E	
Ad4	21.4

^a The self-annealing background of the probe (1.1% in the presence of 4 μg of KB cell DNA per ml) has been subtracted, and the values have been normalized to maximum hybridization (95.2%) with Ad7 DNA. Hybrids were assayed by using hydroxylapatite.

29 human Ad types. The probe hybridized 85 to 99% with Ad3, 11, 14, 16, and 21, but only 6 to 21% with DNAs of the other Ad types. Thus, Ad3, 7, 11, 14, 16, and 21 form a group (group B) with quite homologous transforming sequences that are mainly absent from the DNAs of other Ad types.

Homology of Ad12 (Huie *Eco*RI-C and *Sal*I-C fragments with DNAs of 29 human Ad serotypes. Hybridization results of Ad12 (Huie) *Eco*RI-C fragment with DNAs of 29 Ad types (including 5 other strains of Ad12) are presented in Table 3. Compared to 100% (normalized) hybridization to the homologous Ad12 (Huie) DNA, the probe hybridized from 53 to 68% with DNAs of the various Ad12 strains, 53% with Ad18 DNA, 56% with Ad31 DNA, but <11%

TABLE 3. Hybridization of *in vitro* ^3H -labeled Ad12 (Huie) *EcoRI*-C fragment with DNAs of 29 human Ad serotypes

DNA	% Hybridization ^a
Group A	
Ad12 (strain Huie)	100.0
Ad12 (strain B)	48.1
Ad12 (strain C)	64.3
Ad12 (strain 565)	74.3
Ad12 (strain 015163)	77.8
Ad18	53.0
Ad31	53.1
Group B	
Ad3	8.4
Ad7	2.6
Ad11	3.3
Ad14	5.9
Ad16	7.5
Ad21	7.2
Group C	
Ad1	8.4
Ad2	8.1
Ad5	9.2
Ad6	4.6
Group D	
Ad8	10.5
Ad9	11.4
Ad10	7.7
Ad13	8.9
Ad15	12.9
Ad19	11.2
Ad20	6.0
Ad 22	9.6
Ad23	11.0
Ad24	6.3
Ad26	6.3
Ad27	3.0
Ad28	10.7
Ad29	11.8
Ad30	9.4
Group E	
Ad4	8.4
Other viruses	
SA-7	4.6
SV40 ^b	4.4

^a The self-annealing background of the probe (5.1% in the presence of 4 μg of KB cell DNA per ml) has been subtracted, and the values have been normalized to the maximum hybridization (89.4%) with Ad12 (Huie) DNA. Hybrids were assayed by using hydroxylapatite.

^b SV40, Simian virus 40.

with DNAs of other serotypes.

Table 4 summarizes the hybridization results of Ad12 (Huie) *SalI*-C fragment with DNAs of six other strains of Ad12, with Ad18 and Ad31 DNAs, and with DNAs representing group B (Ad7), group C (Ad2), group D (Ad26), and group E (Ad4). Also tested were DNAs of SA-7, simian virus 40, BK virus, and human papillomavirus type 1. Hybrids were assayed using both

TABLE 4. Hybridization of *in vitro* ^{32}P -labeled Ad12 (Huie) *SalI*-C fragment with DNAs of eight other group A Ad isolates, and representative members of groups B through E

DNA	% Hybridization ^a	
	Hydroxylapatite	S-1 nuclease
Group A		
Ad12 (strain Huie)	100.0	100.0
Ad12 (strain B)	34.6	17.2
Ad12 (strain C)	55.7	26.7
Ad12 (strain 565)	65.1	36.0
Ad12 (strain 572)	70.6	44.3
Ad12 (strain 015163)	67.4	40.2
Ad18	43.7	16.8
Ad31	52.0	20.0
Group B		
Ad7	1.9	2.7
Group C		
Ad2	3.6	0.2
Group D		
Ad26	7.1	2.6
Group E		
Ad4	3.0	0.6
Other viruses		
SA-7	3.0	0.6
BK virus	2.0	-0.2
Human papillomavirus type 1	2.5	1.6
SV40 ^b	2.3	1.3

^a Hybrids were assayed by using both the hydroxylapatite and S-1 nuclease procedures, as indicated. The self-annealing background of the probe (2.0% by hydroxylapatite, 2.1% by S-1 nuclease) has been subtracted and the values have been normalized to the hybridization value with Ad12 (Huie) DNA (92.2% by hydroxylapatite, 65% by S-1 nuclease).

^b SV40, Simian virus 40.

hydroxylapatite and the more stringent S-1 nuclease procedure. When hydroxylapatite was used, essentially the same results were obtained as with the *EcoRI*-C probe. When S-1 nuclease was used, the *SalI*-C probe hybridized from 17 to 44% with DNAs from the Ad12 strains, 17% with Ad18 DNA, and 20% with Ad31 DNA, but did not hybridize with the other viral DNAs.

These results indicate that Ad12, 18, and 31 form a group (group A) of viruses that share common sequences in the early transforming gene block 1 and that these sequences are essentially absent from the genomes of other Ad types. Compared to group C Ads, the group A Ad transforming region is surprisingly heterologous, even among different strains of Ad12. About the same amount of heterology was observed when the entire *in vitro*-labeled Ad12 (Huie) DNA genome was annealed with DNAs of different Ad12 strains and with Ad18 and Ad31 (Green et al., Virology, in press).

Determination of T_m of duplexes formed between Ad12 (Huie) *EcoRI*-C and DNAs of group A Ads and between Ad5 *HindIII*-G and DNAs of group C Ads. The incomplete hybridization of the Ad12 (Huie) DNA fragment probes with DNAs of group A Ads could indicate that the DNAs contain distinct very homologous and very heterologous regions or that the DNAs contain random base-pair mismatches. To distinguish between these two possibilities, we determined the melting points (T_m) of the duplexes between the *EcoRI*-C probe and the other group A Ad DNAs. Hybrids were loaded onto water-jacketed hydroxylapatite columns and were eluted by increasing the water-jacket temperature in increments. The T_m values are presented in Table 5. The T_m of C-fragment hybrid with homologous Ad12 (Huie) DNA was 83°C, but was 6 to 12°C lower with DNAs of other group A Ads. Figure 3 shows the thermal elution curves of the C-fragment hybrid with Ad12 (Huie) DNA and with Ad12 (strain 01563) and with Ad18 DNAs; the latter hybrids represent the best match and the worst match, respectively, of all the non-Huie hybrids examined. The C-fragment hybrid with Ad12 (Huie) DNA melted over a relatively narrow temperature range (ca. 15°C) compared to the Ad12 (01563) (ca. 19°C) and Ad18 (ca. 29°C) hybrids. The elution profiles were linear, with no obvious plateaus. The results are consistent with randomized sequence divergence between Ad12 (Huie) *EcoRI*-C fragment and DNAs of the other group A Ads, rather than large highly matched and highly mismatched regions.

The T_m 's of the duplexes between the Ad5 *HindIII*-G fragment and other group C Ad DNAs are also presented in Table 5. As expected

TABLE 5. Melting points (T_m) of the duplexes between group A Ad DNAs and Ad12 (Huie) *EcoRI*-C fragment, and group C Ad DNAs and Ad5 *HindIII*-G fragment^a

DNA	T_m (°C)
Ad12 (Huie) <i>EcoRI</i> -C [³ H]DNA	
Ad12 (strain Huie)	83
Ad12 (strain B)	76
Ad12 (strain C)	74
Ad12 (strain 565)	77
Ad18	71
Ad31	74
Ad5 <i>HindIII</i> -G [³ H]DNA	
Ad1	84
Ad2	84
Ad5	84
Ad6	84

^a Representative melting curves are shown in Fig. 3.

FIG. 3. Thermal melting profiles of in vitro-labeled Ad12 (Huie) *EcoRI*-C duplexed with Ad12 (Huie) DNA, and with DNAs of other group A Ads.

from the hybridization results, all duplexes had the same T_m value, 84°C.

DISCUSSION

We have investigated the base sequence homologies between the left end regions of group A, B, and C Ads, by hybridizing in vitro-labeled restriction fragments with DNAs of 29 Ad serotypes. The probes used were the Ad5 (group C) *HindIII*-G fragment (map position 0-7.3), the Ad12 (group A) *EcoRI*-C fragment (map position 0-16) and Ad12 *SalI*-C fragment (map position 0-10.6), and the Ad7 (group B) *XhoI*-C fragment (map position 0-10.8); the probes represent much or all of early gene block 1 including the transforming region. The results indicate that group A, B, and C Ads each have unique sets of transforming base sequences that are mostly absent from the DNAs of serotypes outside the group.

Ad5 *HindIII*-G hybridized 98 to 100% with DNAs of Ad1, 2, and 6, but only 1 to 15% with other Ad DNAs. The thermal stability of the hybrid was identical with DNAs of Ad1, 2, 5, or 6. Therefore, the transforming regions of group C Ads are very homologous.

Ad7 *XhoI*-C hybridized 85 to 99% with DNAs of Ad3, 11, 14, 16, or 21, but only 6 to 21% with non-group B Ad DNAs. Therefore, the sequences containing early gene block 1 (transforming region) of group B Ads are also very homologous, although less so than group C Ads.

Ad12 (Huie) *EcoRI*-C and *SalI*-C fragments hybridized 53 to 68% to DNAs of group A Ads, but <11% to DNAs of other types. When *S*-1 nuclease was used, the *SalI*-C fragment hybridized 17 to 40% with other group A Ads, but did not hybridize significantly to non-group A Ads.

These S-1 nuclease values may provide a more stringent measure than the hydroxylapatite values of the "true" homology of the hybrids, because S-1 nuclease digests all nonhybridized sequences, whereas hydroxylapatite scores nonhybridized portions of the probe linked to hybridized portions. The T_m 's of the hybrids between Ad12 (Huie) *EcoRI*-C and the heterologous group A Ads were 6 to 12°C lower than the T_m with homologous Ad12 (Huie) DNA. Mismatched base pairs have been estimated to lower the T_m about 0.7°C per 1% altered pairs (36). Thus, group A Ads also appear to have a unique set of related early gene block 1 base sequences, but the relatedness is not nearly as striking as with the group C or group B Ads. (We cannot be certain of the actual homology between the group A Ad transforming regions, because this could be as little as 1% of the genome, sufficient to code a 10,500-dalton polypeptide [see below]. However, our data are good evidence that the transforming regions of group A Ads are related.) The heterology of the group A Ads gene block 1 region is typical of the general heterology of the entire genomes of these viruses (9; Green et al., *Virology*, in press).

The transforming sequences of group A, B, and C Ads are essentially absent from the DNAs of group D Ads. Since group D Ads transform cells, it follows logically that the transforming sequences of group D Ads differ from those of group A, B, or C Ads. We do not know whether all group D types have homologous transforming sequences, although this is probably the case because the complete genomes of group D types are 88 to 98% homologous (hybrids assayed using S-1 nuclease). To our knowledge, Ad4 (group E) has not been shown to transform cells or to induce tumors (2). But if Ad4 does have transforming sequences, they must differ from those of group A, B, or C Ads.

We have no data on the putative transforming sequences of serotypes not included in our study. Ad17 and Ad25 are in group D and therefore are presumably group D Ad-specific transforming sequences. Reports have appeared claiming the isolation of Ad32, Ad33 (1), Ad34 (23), and Ad35 (49). We have not investigated these isolates.

Our data are consistent with earlier hybridization experiments which demonstrated that rodent cells transformed by group A, B, and C Ads synthesize only group-specific RNA sequences (16). Based on the assumption that the transforming region codes for a protein(s) in transformed cells that represents the "T-antigen" (at least in part), our data also explain why group A, B, C, and D Ads each induce specific T-antigens (24). It would appear that grouping Ads

on the basis of transforming region homology, T-antigen, and tumorigenicity conforms to the grouping on the basis of whole genome homology.

Considering that group A, B, and C Ads, probably group D Ads, and possibly Ad4 (group E), have unique transforming sequences, interesting questions are whether and how this is reflected in the different oncogenic properties of these viruses. Again, assuming that these regions encode a protein(s) that maintains and initiates cell transformation, do the proteins of each group differ in function or merely in efficiency? That is, do all Ads transform cells by the same mechanism or by different mechanisms? Our hybridization data may suggest that the transformation proteins of each Ad group are biologically different. On the other hand, because of the degeneracy in the genetic code, an identical protein can be coded by DNAs that differ somewhat in nucleotide sequence. In addition, substitution of some amino acids can probably be tolerated before the function(s) of the transformation protein(s) becomes substantially altered. Therefore, the heterology of the transforming regions of four groups of Ads does not exclude the possibility that they code for proteins with similar roles in virus replication and cell transformation. Candidate transformation proteins have been identified of 15,000 (11, 32), 38,000 to 50,000 (32), and 53,000 to 58,000 (11, 30) daltons coded by Ad2 (Ad5), and 40,000 to 65,000, 16,500, and 10,500 daltons coded by Ad12 (W. Wold, G. Chinnaduari, M. Green, and S. Mak, *Virology*, in press). Thus, the transforming regions of group A and C Ads code polypeptides of similar sizes, although the functional relationships between these polypeptides is unknown.

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