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Transforming Genes Among Three Different Oncogenic Subgroups of Human Adenoviruses Have Similar Replicative Functions

JOHN S. BRUSCA AND G. CHINNADURAI*

Institute for Molecular Virology, St. Louis University Medical Center, St. Louis, Missouri 63110

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We have examined the functional similarity of the transforming genes for replicative functions among three different subgroups of human adenoviruses (A, B, and C), using mutant complementation as an assay. A host range deletion mutant (*dl*201.2) of Ad2 (nononcogenic subgroup C) lacking about 5% of the viral DNA covering two early gene blocks (E1a and E1b) involved in cellular transformation was isolated and tested for its ability to replicate in nonpermissive KB cells in the presence of Ad7 (weakly oncogenic group B) or Ad12 (highly oncogenic group A). The complementation of the mutant defect was demonstrated by cleaving the viral DNA extracted from mixed infected cells or the DNA extracted from purified virions from mixed infected cells with restriction endonuclease *Bam*HI, which produces a different cleavage pattern with the DNA of each serotype. It was found that the defects in E1a plus E1b of *dl*201.2 could be complemented by Ad7 and Ad12, indicating that these genes in Ad2, Ad7, and Ad12 have similar functions during productive infection.

Human adenoviruses have been grouped by Huebner and his colleagues into three "oncogenic subgroups" (A, B, and C), based on their oncogenic potential for newborn hamsters (17, 18). Members of group A (Ad12, 18, and 31) are highly oncogenic, members of group B (Ad3, 7, 11, 14, 16, and 21) are weakly oncogenic, and members of group C (Ad1, 2, 5, and 6) are nononcogenic. Human adenoviruses have also been arranged into "T-antigen subgroups" which are in accordance with the oncogenic subgroups, based upon the cross-reactivity of T-antigens (17). Green and co-workers (22) have recently reported the existence of five distinct DNA homology groups, A through E, which are consistent with the classification based on tumorigenicity. Members of the three oncogenic subgroups (A, B, and C) transform tissue culture cells in vitro (12). With the development of the calcium technique for DNA transfection by Graham and van der Eb (11), it has been possible to localize the transforming segment of the viral genome by transfection with restriction fragments of the viral DNA. These segments are located at the left termini of the linear genomes of Ad2 and Ad5 DNA (8, 29), Ad12 DNA (25), and Ad7 DNA (24, 29). In the case of Ad5, transformation-defective host range (*hr*) mutants which grow well on an Ad5-transformed human embryonic kidney cell line which expresses the left 11% of Ad5 genome (293 cells) (10), but not on HeLa or KB cells, have been isolated and physically mapped within the left 11% of the viral genome (7, 9, 15, 19). This region is comprised of two transcriptional units, E1a and E1b (6). E1a maps between map position (mp) 1.3 and 4.4 and E1b between mp 4.5 and 11.2 (1, 4). It has been suggested, based on genetic studies with Ad5 *hr* mutants, that E1a may be involved in the maintenance of cellular transformation and that E1b may be involved in the initiation of cellular transformation (9).

The transforming segments of the viral DNA from members of the three subgroups do not have significant homology as measured by DNA-DNA hybridization studies, but members of the same subgroup show extensive homology (21). Recently, the nucleotide sequences of the left 8% of the viral genomes of Ad5 (20, 30), the left 6.8% of Ad12 (27), and the left 4.5% of Ad7 (5) have been determined by direct DNA sequence analysis. These data indicate that the overall organization of the transforming genes in these viruses is quite similar, but the sequences, other than the possible regulatory signals for synthesis of RNA and protein and for RNA splicing, diverge considerably. The predicted transforming proteins contain regions which have similar sequences and other regions which have dissimilar sequences. The predicted amino acid sequence homology in E1a among the three subgroups is about 35%, whereas the amino acid sequences in

E1b of Ad5 and Ad12 diverge considerably (the nucleotide sequence for E1b of Ad7 is not yet available).

In light of these results, we tested whether the replicative functions of the transforming genes among the three subgroups can complement one another during productive infection. For these studies, we used a host range deletion mutant of Ad2 which was isolated in our laboratory and which lacks a segment of the viral genome between mp 2.0 and 7.0 (*dl*201.2) in E1a and E1b. We examined viral DNA synthesis and the production of viral particles of *dl*201.2 in KB cells (nonpermissive for *dl*201.2) in mixed infections with Ad7 wild type (WT) or Ad12 WT. It was found that the Ad2 mutant could be very efficiently complemented by Ad7 or Ad12.

Mutant *dl*201.2 was derived from a spontaneous deletion mutant, *dl*201.1, which in turn was isolated from an insertion mutant of Ad2 (*dp*201) in which the left 3% of the viral genome is duplicated at the normal right end (Brusca and Chinnadurai, manuscript in preparation). Mutant *dl*201.2 has the same deletion (mp 2.0 to 7.0) as the parental *dl*201.1 but has lost the duplicated sequences at the right end. The deletions in *dl*201.1 and *dl*201.2 were mapped by digestion with a number of restriction endonucleases. The *Kpn*I cleavage patterns are shown in Fig. 1. DNAs from *dl*201.1 and *dl*201.2 did not contain the *Kpn*I-G (mp 0 to 5.8) or the *Kpn*I-B (mp 5.8 to 23.5) fragments. Instead, a new fragment which migrated more slowly than B, designated B′, was observed. The B′ fragment had an increase in size of about 0.8% (300 base pairs) relative to B and arose from the fusion of G and B fragments by a deletion of about 5% of the viral genome spanning the *Kpn*I cleavage site at mp 5.8. The mapping of the mutants obtained by using a variety of restriction enzymes is summarized in Fig. 2. The results show that the deletion of *dl*201.1 and *dl*201.2 covers both E1a and E1b.

The host range properties of these mutants were tested on 293 and KB cells. 293 cells are human embryonic kidney cells transformed by sheared Ad5 DNA which retain and express the left 11.5% (E1a and E1b) of the Ad5 genome (10). As seen in Table 1, these mutants failed to form plaques on KB cells but formed plaques on 293 cells efficiently, indicating that the Ad5 genes in 293 cells complemented the growth of these mutants.

For our complementation studies described below, we used *dl*201.2. This mutant was also tested for complementation of Ad5 *hr* deletion mutants *dl*312 (mp 1.5 to 4.0), which maps in E1a, and *dl*313 (mp 3.5 to 10.5), which maps in E1b (19). It was found that *dl*201.2 did not complement *dl*312 or *dl*313, confirming that the *dl*201.2 lesion is in E1a and E1b (data not shown).

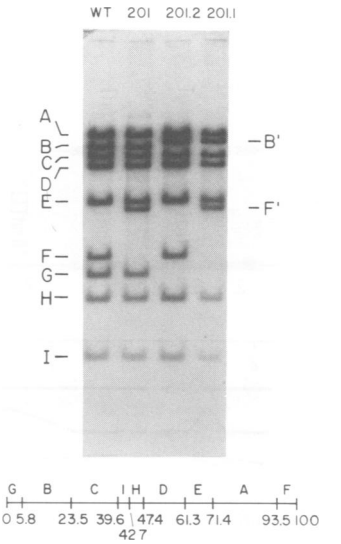

FIG. 1. *Autoradiogram of an agarose gel showing KpnI cleavage patterns of DNAs from Ad2 WT and Ad2 mutants.* ^{32}*P-labeled viral DNA was selectively extracted from 293 cells infected with Ad2 WT or with various mutants as described previously (2) and cleaved with KpnI, and the fragments were separated by electrophoresis on 1% agarose slab gels, dried, and autoradiographed. The KpnI cleavage map of Ad2 WT (28) is shown below the autoradiogram. The fragments designated by a prime are those having the deletion and insertion mutations.*

We tested whether the E1a and E1b functions deleted in *dl*201.2 could be complemented with Ad7, which belongs to subgroup B. Ad7 WT was coinfected with Ad2 WT or *dl*201.2 in KB cells; viral DNA was labeled with ^{32}P and isolated from virus purified by banding in CsCl. Viral DNA was also prepared from infected cells by the Hirt extraction (16). These DNAs were cleaved with *Bam*HI which cleaves Ad7 DNA nine times (32) and Ad2 DNA three times (23) (Fig. 3). These cleavage patterns are characteristic of the two serotypes from mixed infected cells, and each Ad DNA could be easily distinguished.

*dl*201.2 did not synthesize viral DNA in KB cells (Fig. 3, lane 2), as expected. In cells infected with Ad2 WT and Ad7 WT, synthesis of both Ad7 and Ad2 DNAs was observed as revealed by their respective *Bam*HI restriction patterns (Fig. 3, lanes 6 and 7 compared with lanes 3 and 5). Similarly, in cells infected with Ad7 WT and

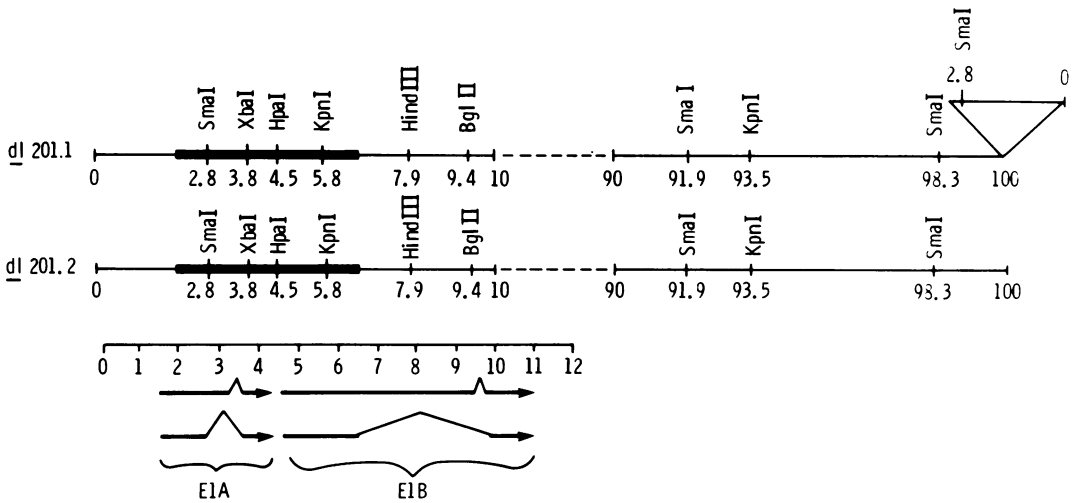

FIG. 2. *Restriction mapping of dl201.1 and dl201.2 DNAs. The left and right 10% of the viral genome is shown. In the right 10%, only the KpnI and SmaI cleavage sites are shown. The thick lines indicate the segment of viral DNA deleted. The wedge in dl201.1 indicates the insertion. The major mRNA species transcribed from Ad2 WT E1a and E1b (1) are shown below dl201.2.*

TABLE 1. *Host range of dl201.1 and dl201.2*[a]

Virus	Virus yield (PFU/ml)	
	293	KB
Ad2 WT	7.5×10^7	1.3×10^8
*dl*201.1	1×10^7	$<10^1$
*dl*201.2	9×10^6	$<10^1$

[a] 293 cells were infected with Ad2 WT or the deletion mutants at about 1 PFU per cell and incubated at 37°C until complete cytopathic effect was observed. Infected cells were frozen, thawed, sonicated, clarified, titrated on 293 or KB cells, and treated for plaque assay (3).

*dl*201.2, synthesis of both DNAs was seen (Fig. 3, lane 8 compared with lanes 4 and 5). Complementation also permitted the production of virus particles of *dl*201.2 as revealed by the DNA extracted from virions purified from mixed infected cells (Fig. 3, lane 9 compared with lanes 4 and 5). Similar results were also obtained when the DNAs were cleaved with *Eco*RI, which cleaves the two viral DNAs differently (data not shown). These results show that Ad7 genes can complement the E1a and E1b replicative functions deleted in *dl*201.2.

Complementation experiments similar to those described for Ad7 were carried out with Ad12 (group A). In these experiments, restriction endonuclease *Bam*HI was used to distinguish between Ad2 and Ad12 DNA. *Bam*HI cleaves Ad2 DNA three times and Ad12 DNA eight times (28). In cells mixed-infected with Ad12 WT and Ad2 WT, synthesis of both viral DNAs was observed (Fig. 4, lanes 6 and 7). In mixed infections with Ad12 WT and *dl*201.2, the viral DNA as well as viral particles synthesized were mainly that of *dl*201.2, and very little Ad12 DNA was detected (Fig. 4, lanes 8 and 9). These results demonstrated that Ad12 complemented the defects of *dl*201.2.

We have shown that a deletion mutant of nononcogenic Ad2 (group C) defective in early transforming genes E1a (mp 1.3 to 4.4) and E1b (mp 4.5 to 11.5) could be complemented by weakly oncogenic Ad7 (group B) and highly oncogenic Ad12 (group A) under conditions of productive infection. DNA homology studies of the transforming segments (mp ~0 to 8.0) revealed very little homology among the three subgroups (21). The DNAs of the transforming regions of Ad5 (closely related to Ad2), Ad7, and Ad12 have recently been sequenced (5, 20, 27, 30). The results revealed conservation of some of the E1a sequences, providing about 35% homology in amino acid sequences among the three serotypes. The amino acid sequences in E1b of Ad5 and Ad12 (only part of the DNA sequences between mp 4.5 and 6.8 are known) diverge greatly. It is interesting to note that although considerable divergence in nucleotide and amino acid sequences exists among the three serotypes, the replicative functions of the proteins encoded in the transforming region may be conserved in these viruses, as indicated by the ability of Ad7 and Ad12 to complement Ad2 *dl*201.2.

The observed complementation is not due to recombination between the two serotypes be-

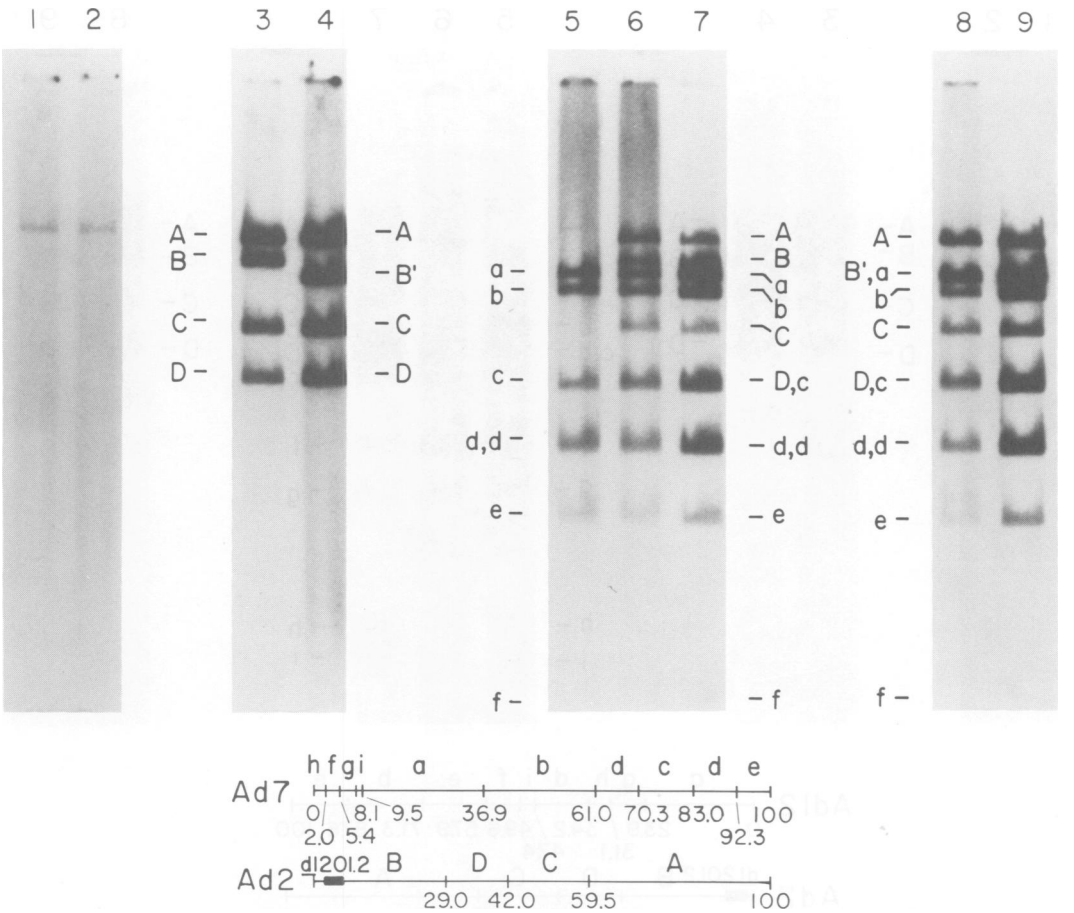

FIG. 3. *Autoradiogram of an agarose gel showing BamHI cleavage patterns of Ad2 and Ad7 DNAs from mixed infected cells.* ^{32}*P-labeled DNAs from uninfected cells, singly infected cells (10 PFU per cell), and mixed infected cells (5 PFU per cell of each virus) were extracted, cleaved with BamHI, and analyzed by electrophoresis on 1% agarose gels. The capital letters represent the Ad2 fragments, and the lower case letters represent Ad7 fragments. B' fragment represents the fragment of dl201.2 in which the deletion is located. The various lanes represent DNA from the following sources: (1) uninfected KB cells; (2) KB cells infected with dl201.2; (3) KB cells infected with Ad2; (4) 293 cells infected with dl201.2; (5) KB cells infected with Ad7; (6 and 7) KB cells infected with Ad2 and Ad7; (8 and 9) KB cells infected with Ad7 and dl201.2. In lanes 7 and 9, the DNAs were extracted from purified virions (13, 14), and in all other lanes, DNAs were extracted by the Hirt method. The BamHI cleavage maps of Ad7 (32) and Ad2 (23) are shown below the autoradiogram. BamHI-g, -h, and -i fragments of Ad7 ran off the gel during electrophoresis.*

cause the restriction pattern in the mixed infection is simply the sum of the patterns of the two infecting virus. It will be interesting to see whether the three serotypes could complement one another in cellular transformation. Such studies will have to wait for the availability of suitable mutants of Ad7 and Ad12. The results presented here indicate it may be possible to isolate host range mutants of Ad7 and Ad12 by using 293 cells, and the results should prove useful for transformation studies.

Intertypic complementation for certain adenovirus genes (whose comparative nucleotide sequences are not yet known) has been reported previously. Williams and co-workers (31) have shown that some *ts* mutants of Ad5, such as hexon-deficient mutants, are complemented by Ad12, but that two early DNA-negative mutants (*ts*36 and *ts*125) and fiber-deficient mutants were not complemented by Ad12. Shimojo and co-workers (26) have shown that some DNA-negative *ts* mutants (*ts*B and *ts*C) of Ad12 can be complemented by an African green monkey cell line transformed by Ad7.

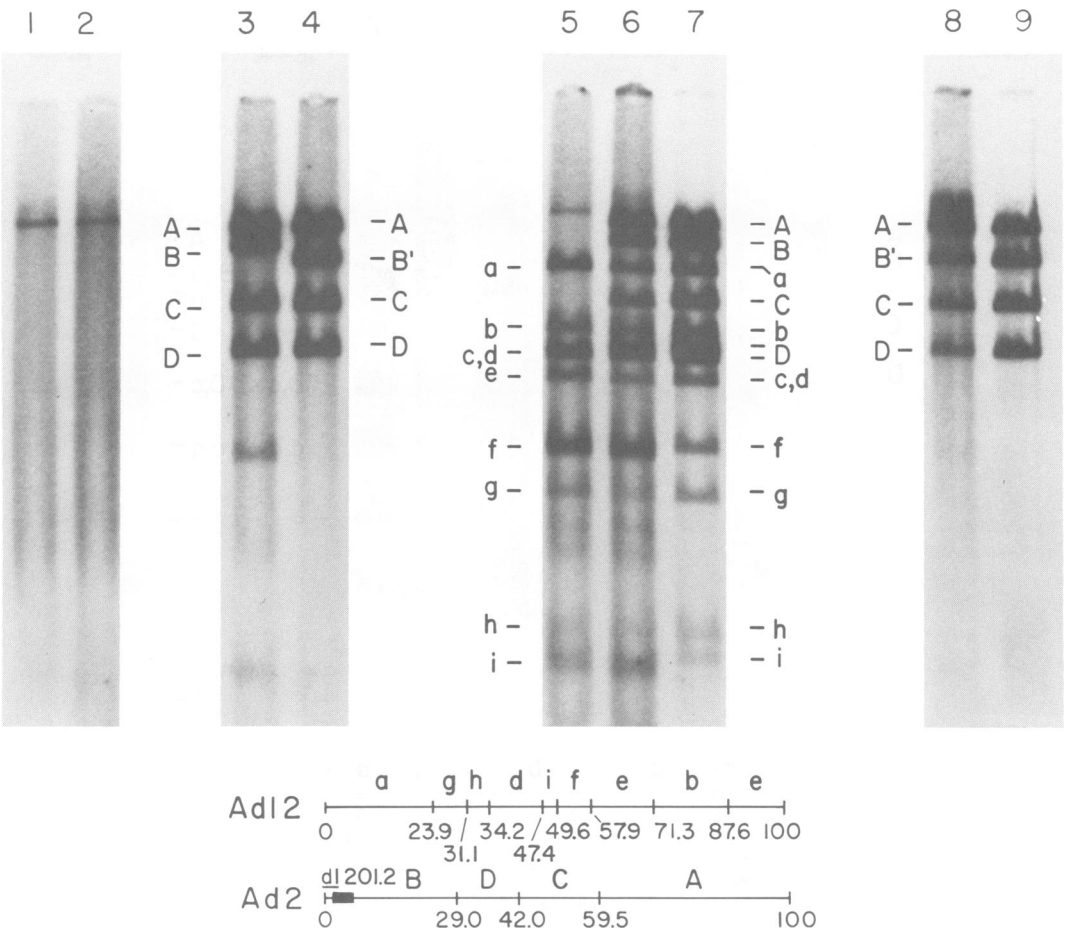

FIG. 4. *Autoradiogram of an agarose gel showing BamHI cleavage patterns of Ad2 and Ad12 DNAs from mixed infected cells.* ^{32}*P-labeled DNA was extracted from infected cells and analyzed as described in Fig. 3. The capital letters represent the Ad2 fragments, and the lower case letters represent Ad12 DNA fragments. B' fragment represents the fragment of dl201.2 in which the deletion is located. The various lanes represent DNA from the following sources: (1) uninfected KB cells; (2) KB cells infected with dl201.2; (3) KB cells infected with Ad2; (4) 293 cells infected with dl201.2; (5) KB cells infected with Ad12; (6 and 7) KB cells infected with Ad2 and Ad12; (8 and 9) KB cells infected with Ad12 and dl201.2. In lanes 7 and 9, the DNAs were extracted from purified virions. In all other lanes, DNAs were extracted by the Hirt method. The BamHI cleavage maps (28) are shown below the autoradiogram.*

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