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Adenovirus *cyt*$^+$ Locus, Which Controls Cell Transformation and Tumorigenicity, Is an Allele of *lp*$^+$ Locus, Which Codes for a 19-Kilodalton Tumor Antigen

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The early region E1b of adenovirus type 2 (Ad2) codes for two major tumor antigens of 53 and 19 kilodaltons (kd). The adenovirus *lp*$^+$ locus maps within the 19-kd tumor antigen-coding region (G. Chinnadurai, Cell 33:759–766, 1983). We have now constructed a large-plaque deletion mutant (*dl*250) of Ad2 that has a specific lesion in the 19-kd tumor antigen-coding region. In contrast to most other Ad2 *lp* mutants (G. Chinnadurai, Cell 33:759–766, 1983), mutant *dl*250 is cytocidal (*cyt*) on infected KB cells, causing extensive cellular destruction. Cells infected with Ad2 *wt* or most of these other Ad2 *lp* mutants are rounded and aggregated without cell lysis (*cyt*$^+$). The *cyt* phenotype of *dl*250 resembles the *cyt* mutants of highly oncogenic Ad12, isolated by Takemori et al. (Virology 36:575–586, 1968). By intertypic complementation analysis, we showed that the Ad12 *cyt* mutants indeed map within the 19-kd tumor antigen-coding region. The transforming potential of *dl*250 was assayed on an established rat embryo fibroblast cell line, CREF, and on primary rat embryo fibroblasts and baby rat kidney cells. On all these cells, *dl*250 induced transformation at greatly reduced frequency compared with *wt*. The cells transformed by this mutant are defective in anchorage-independent growth on soft agar. Our results suggest that the 19-kd tumor antigen (in conjunction with E1a tumor antigens) may play an important role in the maintenance of cell transformation. Since we have mapped the low-oncogenic or nononcogenic Ad12 *cyt* mutants within the 19-kd tumor antigen-coding region, our results further indicate that the 19-kd tumor antigen also directly or indirectly plays an important role in tumorigenesis of Ad12. Our results show that the *cyt*$^+$ locus is an allele of the *lp*$^+$ locus and that the *cyt* phenotype may be the result of mutations in specific domains of the 19-kd tumor antigen.

Earlier we isolated several mutants of adenovirus type 2 (Ad2) (group C) that produce large clear plaques on human KB cells. These mutants were designated large-plaque (*lp*) mutants based on their plaque morphology (5, 6). Two of the seven *lp* mutants that we isolated have been mapped within the 19-kilodalton (kd) tumor antigen-coding region of the early gene block E1b. A series of mutants of Ad12 (group A), designated *cyt* mutants, were isolated by Takemori et al. (23). The *cyt* mutants produce large clear plaques on human embryonic kidney (HEK) cells and were found to be cytocidal on HEK and KB cells. The cytopathic effect (CPE) of the *cyt* mutant-infected cells is marked by extensive cellular destruction, whereas *wt*-infected cells are rounded and aggregated without cell lysis. The *cyt* mutants are generally deficient in transformation (18, 23) and are low-oncogenic or nononcogenic in newborn hamsters (23). The Ad2 *lp* mutants are also generally transformation deficient, but most of them are not cytocidal (see below). The relationship between the *lp*$^+$ locus of Ad2 and the *cyt*$^+$ locus of Ad12 is not known.

Now we have constructed a deletion mutant (*dl*250) of Ad2 that has a specific defect in the early region E1b. By intertypic complementation we show that the *cyt*$^+$ phenotype maps within the *lp*$^+$ locus that codes for the 19-kd tumor antigen. We have further extended our previous results on the role of the 19-kd tumor antigen in cell transformation on three different types of rat cells. Our present results indicate that this protein may play a role in the maintenance of the fully transformed phenotype of cells.

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MATERIALS AND METHODS

Cells and virus. Human KB cells were grown in monolayer cultures with Dulbecco modified minimal essential medium (MEM) containing 10% fetal calf serum or in suspension with Joklik modified MEM containing 5% horse serum. CREF cells were gifts from P. Fisher. Rat embryo fibroblasts (REF) were prepared from 2-week-old Fisher rat embryos. Both CREF and REF cells were grown by using Dulbecco modified MEM containing 10% fetal calf serum. Baby rat kidney cells were prepared from 1-week-old Chester Beatty rats and were grown by using α-MEM containing 10% fetal calf serum. Human A549 cells were grown in monolayers with Dulbecco modified MEM containing 10% fetal calf serum. Ad2 *lp* (*lp*1-5) mutants have been described by Chinnadurai (5). Ad12 *wt* (strain 1131) and the *cyt* mutants have been described by Takemori et al. (23).

Construction of Ad2 deletion mutant *dl*250. A deletion between map positions (mp) 4.96 (*Sst*I site) and 5.36 (*Bst*EII site) was introduced into a DNA fragment from the left 8% of the viral genome cloned in pBR322 (pGC212). The plasmid DNA was cleaved with restriction endonucleases *Sst*I and *Bst*EII, and 3′ and 5′ producing ends were blunt-ended with T4 DNA polymerase at 15°C for 1 h in the presence of four deoxyribonucleotides (19), circularized with T4 ligase, and cloned in *Escherichia coli* HB101. The plasmid DNA with

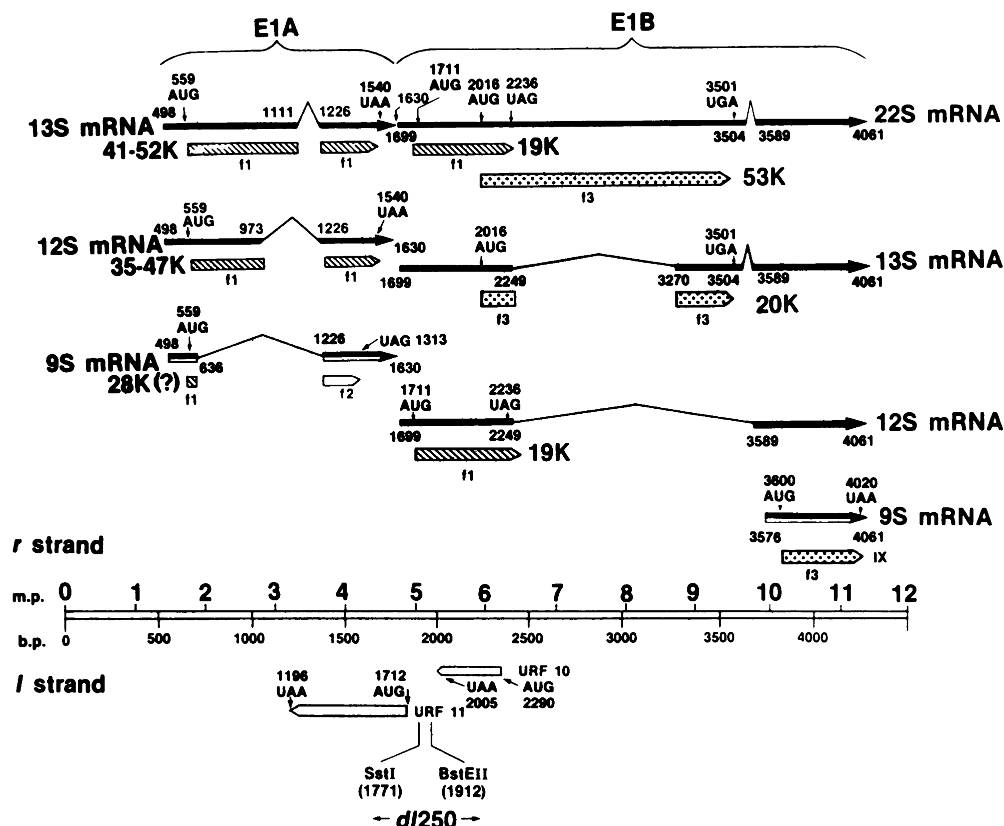


FIG. 1. Organization of the transforming early gene blocks E1a and E1b of Ad2. This figure is based on the DNA sequence analysis of Gingeras et al. (11) and Virtanen and Pettersson (25) and the protein sequence data of Anderson et al. (2). On each mRNA, the cap site, the polyadenylate addition site, and the splice donor and acceptor sites are marked with the base number. The coordinates of the deletion mutation (*SstI* and *BstEII* sites) are marked. The mRNAs shown by solid bars (▬) are expressed at early times, and the mRNAs shown by half-solid, half-open bars (▬) are expressed at late times. bp, Base pairs.

the deletion was used in the marker rescue of Ad2 E1a-E1b host-range deletion mutant *dl*201.2 (4) essentially as described by Chinnadurai (5). The resultant deletion mutant virus was isolated and screened as described by Chinnadurai et al. (6).

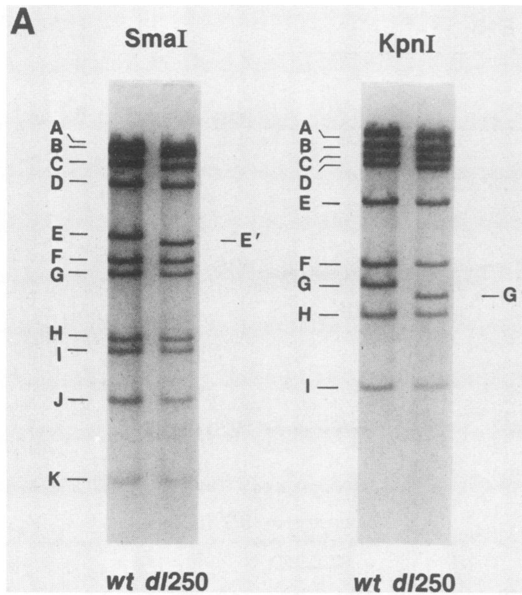
Pulse-labeling of proteins and immunoprecipitation. KB cells in 60-mm dishes were infected with either Ad2 *wt* or *dl*250 for 36 h in the presence of cytosine arabinoside as described by Gaynor et al. (10). Thirty-six hours after infection cells were labeled for 4 h with [^{35}S]methionine (100 μCi/ml). Whole-cell extracts were prepared from infected cells, and the E1b-coded proteins were immunoprecipitated with antibodies directed against synthetic peptides from the N terminus of the 53- and 19-kd tumor antigens (13). The rabbit sera raised against these peptides were gifts from Maurice Green.

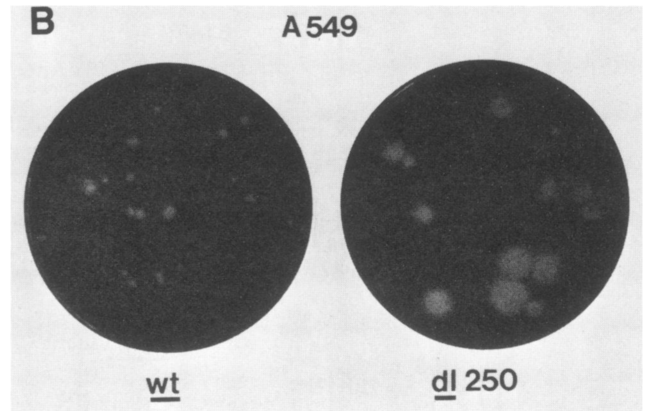
Cell transformation. Confluent cultures of CREF and REF cells were trypsinized and plated at about 1×10^5 to 5×10^5 cells per 25-cm^2 bottle. Two to four hours after plating, cells were infected with Ad2 *wt* or *dl*250 at 1 or 5 PFU per cell. Infected cells were maintained by using Ca^{2+}-free Dulbecco modified MEM containing 10% fetal calf serum for 6 to 8 weeks. Transformation of BRK cells was carried out as described by Mak and Mak (18). Cells were fixed after the incubation period and stained with Geimsa, and the transformed foci were counted.

RESULTS

Construction of Ad2 mutant *dl*250. The organization of the early gene blocks E1a and E1b (reviewed in reference 8) is shown in Fig. 1. The *r* strand of the E1b region has been shown to code for two major tumor antigens. A 53-kd tumor antigen is coded by a 22S mRNA. A second tumor antigen of 19-kd is coded by both the 22S mRNA and a 13S mRNA. In addition to these tumor antigens, a third tumor antigen of ca. 20-kd which is related to the 53-kd tumor antigen has also been identified (2, 12). Apart from these three tumor antigens, the late virion component protein IX and the E1a polypeptides are encoded in the *r* strand. The *l* strand of the E1 region also contains two open reading frames (URF10 and URF11), the protein products of which are not fully identified (11).

Restriction endonucleases *SstI* and *BstEII* each have a single site within the left 8% of the viral genome at mp 4.96 and 5.36, respectively. These sites are located uniquely within the 19-kd tumor antigen-coding region (see Fig 1). We introduced a deletion mutation of 145 base pairs (bp) from positions 1768 to 1912 (i.e., between the *SstI* and *BstEII* sites) in plasmid pGC212 which contains the left 8% of the viral genome (5) as described above. The deletion mutation in the plasmid DNA was confirmed by direct DNA sequence analysis (20) between positions 1569 and 1966. The mutagen-





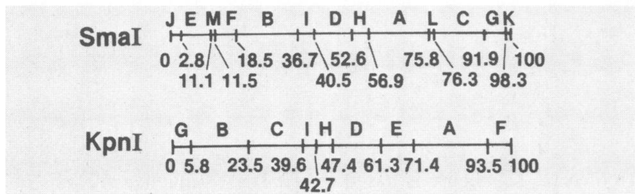


FIG. 2. Isolation of mutant *dl*250. (A) Restriction endonuclease cleavage patterns of Ad2 *wt* and *dl*250 DNA. ^{32}P-labeled viral DNA was selectively extracted from KB cells infected with virus as described by Chinnadurai et al. (6). DNA was digested with the restriction endonucleases, and the fragments were resolved by electrophoresis on a 1.4% agarose gel, which was dried and autoradiographed. The cleavage maps of the restriction endonucleases are given below the autoradiograms. (B) Plaque morphology of Ad2 *wt* and *dl*250 on A549 cells. Cells were stained with neutral red 10 days after infection and photographed on day 15.

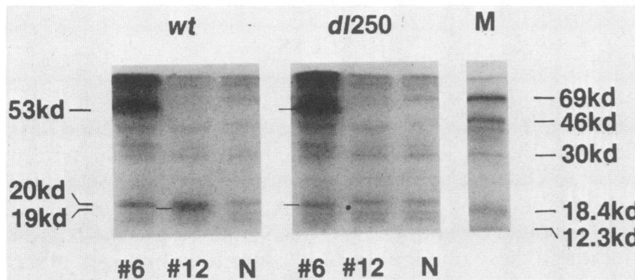


FIG. 3. Immunoprecipitation of E1b-coded tumor antigens. Immunoprecipitations were carried out with whole-cell extracts prepared from infected cells essentially as described (14, 29). The antibodies have been described by Green et al. (13). Lanes: M, molecular weight markers; N, normal serum; #6, peptide 6 antiserum (specific for 53- and 20-kd tumor antigens); #12, peptide 12 antiserum (specific for 19-kd tumor antigen).

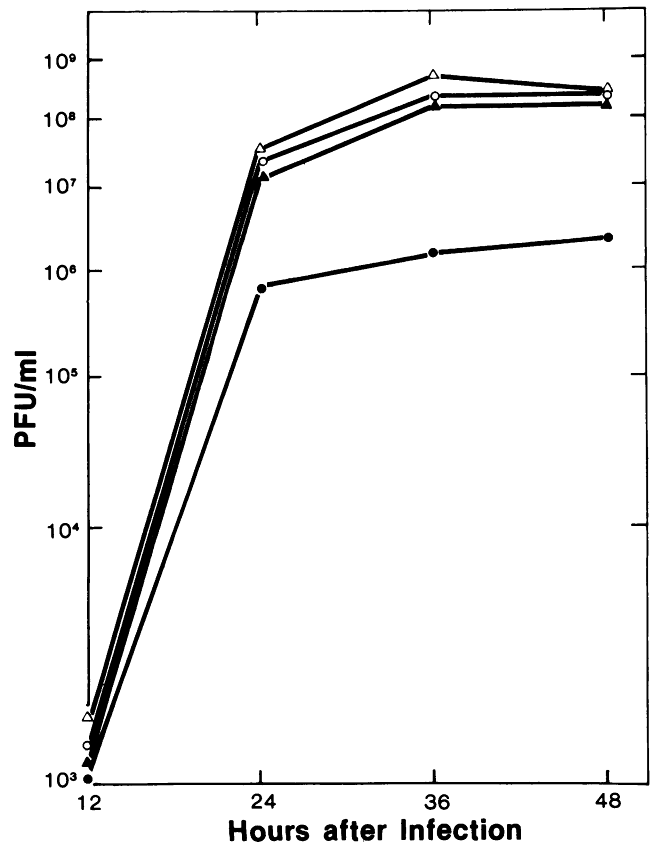


FIG. 4. Growth curve of Ad2 *wt* and *lp* mutants. KB cells were infected at a multiplicity of 5 PFU per cell. Infected cells were frozen at various times, and the progeny virus was titrated by plaque assay on KB cells. Symbols: ○, *wt*; ●, *dl*250; ▲, *lp*3; △, *lp*5.

ized plasmid DNA was used for marker rescue of the E1a-E1b deletion mutant *dl*201.2 in human KB cells as described earlier (5). The mutant plasmid readily rescued mutant progeny virus at a frequency comparable with that of *wt* plasmid pGC212. These results indicate that the mutant lacking these 145 bp that specifically affects the E1b 19-kd tumor antigen is viable in human KB cells. This mutant is designated *dl*250.

To confirm that the deletion mutation is indeed located near the left end of the viral genome and that the mutant does not have detectable alterations in other regions, the ^{32}P-labeled mutant and *wt* DNA were cleaved with restriction endonucleases *Sma*I or *Kpn*I. When the viral DNA was cleaved with *Sma*I, DNA from the mutant *dl*250 did not produce fragment *Sma*I-E (mp 2.8 to 11.1) but instead produced a smaller fragment designated E′ (Fig. 2A). When the viral DNA was cleaved with *Kpn*I, mutant viral DNA did not produce fragment *Kpn*I-G (mp 0 to 5.8) but instead produced a smaller fragment designated G′. The fragments *Sma*I-E′ and *Kpn*I-G′ of the mutant *dl*250 are smaller by ca. 150 bp compared with *wt* fragments. The restriction fragments from other regions of the viral genome did not reveal any alterations, indicating that the deletion mutation is indeed located within the left 2.8 to 5.8% of the viral genome. No other detectable alteration was observed in the rest of the genome.

The plaque morphology of Ad2 *wt* and *dl*250 was examined on human KB cells and A549 cells. As expected from earlier studies in which we mapped the Ad2 *lp* mutants within the 19-kd tumor antigen-coding region (5), mutant *dl*250 also produces large clear plaques, compared with small fuzzy-edged *wt* plaques on KB and A549 cells. The plaque morphology of Ad2 *wt* and *dl*250 on A549 cells is shown in Fig. 2B. In both KB cells and A549 cells, the *lp* morphology was very pronounced ca. 10 to 15 days after infection. Several independent plaque isolates revealed the same plaque morphology and genome structure.

Analysis of E1b proteins coded by *dl*250. The DNA sequence analysis (11) and the N-terminal amino acid sequence analysis (1) have shown that the synthesis of 19-kd tumor antigen is initiated at position 1711. According to the published DNA sequence analysis of the Ad2 DNA, the mutation in *dl*250 would result in the synthesis of the correct first 19 N-terminal amino acids, followed by an additional 28 missense amino acids due to a frameshift at the end of the deletion.

To examine the synthesis of the E1b-coded tumor antigens in cells infected with *dl*250 in comparison with cells infected with Ad2 *wt*, cells were labeled with [^{35}S]methionine, and the E1b proteins were analyzed by immunoprecipitation. The 53- and 20-kd tumor antigens were immunoprecipitated by an antiserum raised against an N-terminal peptide (peptide 6) from the 53-kd tumor antigen. The 19-kd tumor antigen was immunoprecipitated by an antiserum raised

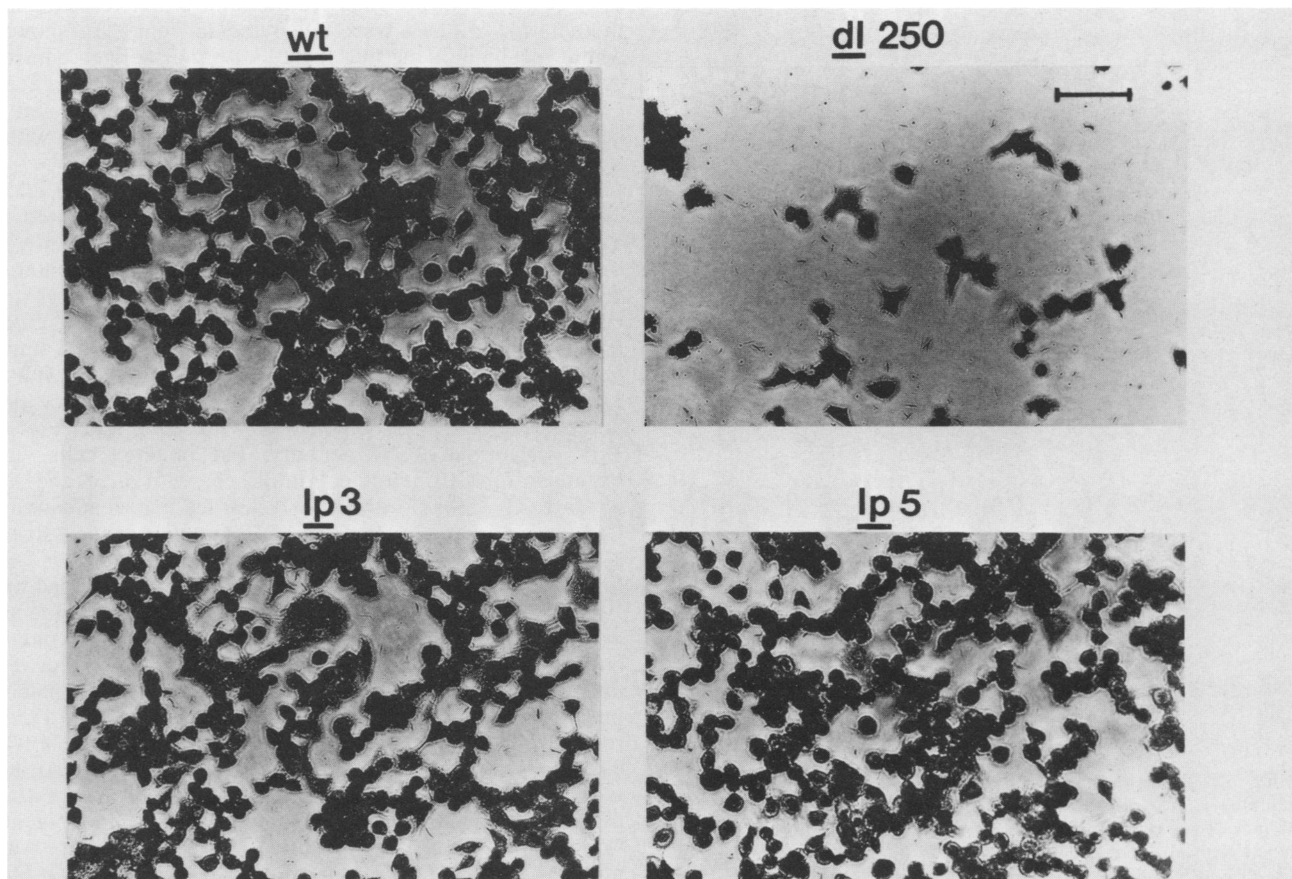


FIG. 5. CPE of KB cells infected with Ad2 *wt* or *lp* mutants. Cells were infected at 30 PFU per cell, fixed at 24 h after infection and stained with Giemsa. Photographs were taken by using a Nikon inverted stage microscope. Bar, 0.1 mm.

against a peptide (peptide 12) from the N terminus of the 19-kd tumor antigen. These antisera and the peptides have been described in detail by Green et al. (13).

Mutant *dl*250 induced both the 53- and the 20-kd tumor antigens (lane *dl*250 #6) to *wt* (lane *wt* #6) levels (Fig. 3). On the other hand, mutant *dl*250 did not induce the synthesis of the 19-kd tumor antigen (lane *dl*250 #12, marked by a dot), although a significant amount of this protein was synthesized in *wt*-infected cells (lane *wt* #12). Although we used antibodies directed against the N terminus of the 19-kd tumor antigen, we could not detect the predicted mutant protein that is 47 amino acids long. Presumably the mutant polypeptide is unstable in the infected cells, or it is not recognized by the antibodies.

Growth characteristics of *lp* mutants. Although mutant *dl*250 is viable (i.e., produces plaques), we consistently obtained reduced yields of the virus from infected cells. This prompted us to examine the growth characteristics of *dl*250

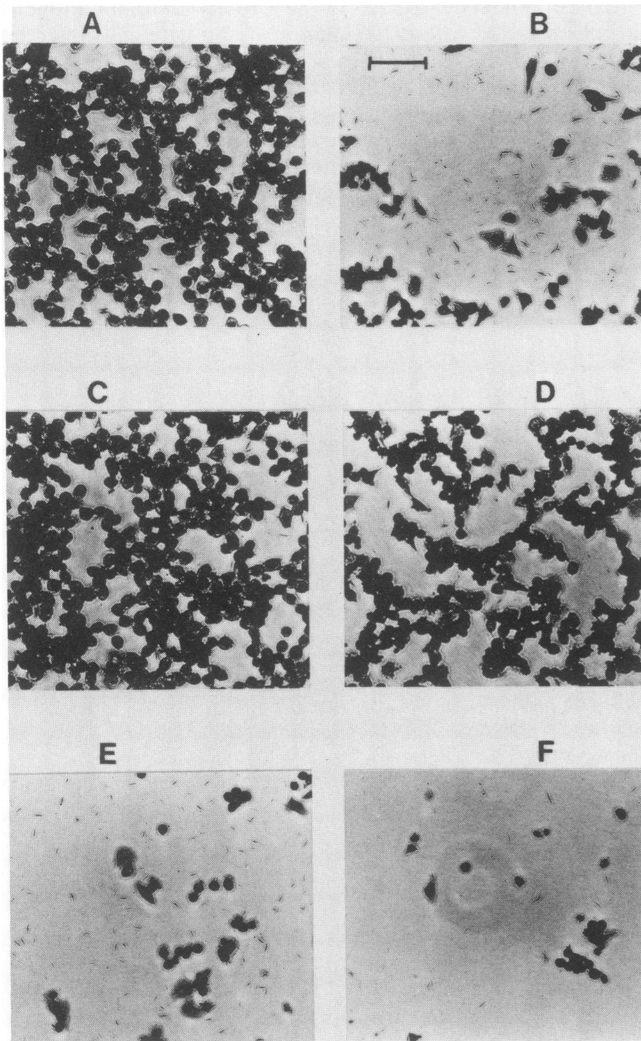


FIG. 6. Complementation patterns of Ad2 *lp* mutants and Ad12 *cyt* mutants. KB cells were singly infected at a multiplicity of 30 PFU per cell or doubly infected at a multiplicity of 15 PFU per cell of each virus. Infected cells were fixed, stained, and photographed as described in the legend to Fig. 5. (A) Ad2 *wt*; (B) *dl*250; (C) Ad2 *wt* plus *dl*250; (D) Ad12 *wt* (strain 1131) plus *dl*250; (E) *dl*250 plus Ad12 *cyt*62; (F) *dl*250 plus Ad12 *cyt*68.

TABLE 1. Transformation of rat cells by Ad2 *wt* and *dl*250

Virus	No. of foci per flask[a] with:						
	CREF (PFU/cell)		REF (PFU/cell)		BRK (virus particles/cell)		
	1	5	1	5	1.5	6	12
Ad2 *wt*	15.2	42.0	5.0	8.2	20	13	4.3
*dl*250	3.0	3.6	0.8	1.4	0	0	2.5
Control	1.8	1.8	0.3	0.3	0	0	0

[a] Average of 5 to 10 flasks.

in comparison with other *lp* mutants and Ad2 *wt*. These results are presented in Fig. 4. Mutant *dl*250 appears to be partially defective and produces amounts of progeny virus that are about 2 orders of magnitude lower than those of the *wt* (on KB cells), whereas mutants *lp*3 and *lp*5 produce *wt* levels of progeny virus.

CPE of *dl*250. Takemori et al. (23) isolated a series of Ad12 mutants designated the *cyt* mutants. The *cyt* mutants produce large clear plaques on HEK cells. These mutants are cytocidal in human KB and HEK cells, causing extensive cellular destruction compared with *wt* virus, which produces typical adenovirus CPE (i.e., the infected cells are rounded and aggregated without cell lysis). Since the *cyt* mutants and *lp* mutants resemble each other in their plaque morphology, we examined whether *dl*250 and other *lp* mutants are cytocidal on KB cells. Mutant *dl*250 caused extensive cellular destruction of infected KB cells, whereas mutants *lp*3 and *lp*5 produced a typical *wt* CPE (Fig. 5). Mutant *lp*2 was found to be cytocidal, although not to the same extent as *dl*250, and mutants *lp*1 and *lp*4 were not cytocidal (data not shown). These results indicate that the 19-kd tumor antigen controls both the plaque morphology and the CPE of the infected cells. Our results also indicate that the *lp* phenotype can be separated from the *cyt* phenotype and that both map within the 19-kd tumor antigen-coding region.

Complementation analysis of Ad12 *cyt* mutants and *dl*250. To determine whether the Ad12 *cyt* mutants isolated by Takemori et al. (23) also map within the 19-kd tumor antigen-coding region, we carried out an intertypic complementation analysis. It is known that the E1 region of Ad12 can complement Ad2 and Ad5 E1 mutants (4, 21, 26). Therefore, if the Ad12 *cyt* mutants also map within the 19-kd tumor antigen-coding region, mixed infections will not result in complementation, i.e., the *wt* CPE (cyt^+) will not be produced. KB cells were coinfected with *dl*250 and Ad2 *wt*, Ad12 *wt*, or Ad12 *cyt* mutants. The infected cells were examined for CPE. Coinfection of cells with *dl*250 and Ad2 *wt* (Fig. 6C) or Ad12 *wt* (Fig. 6D) resulted in a *wt* adenovirus CPE (cyt^+), indicating that the *dl*250 mutation is recessive to Ad2 *wt* and can be complemented by the Ad12 *wt* gene function. On the other hand, when *dl*250 was coinfected with Ad12 *cyt* mutants *cyt*62 (Fig. 6E) or *cyt*68 (Fig. 6F), infected cells showed a *cyt* CPE, indicating that these mutants did not complement *dl*250. The lack of complementation between *dl*250 and Ad12 *cyt* mutants is not due to a possible dominant phenotype of Ad12 mutants because Ad2 *wt* or Ad12 *wt* readily complements *cyt*62 and *cyt*68 (data not shown). These results show that the Ad12 *cyt* mutations also map within the 19-kd tumor antigen-coding region and that the cyt^+ locus is an allele of the lp^+ locus. Earlier complementation analysis with Ad5 *hr*II mutants showed that these Ad12 mutants are not defective in the 53-kd tumor antigen (16). Recently, we (S.M.) have shown that Ad12 *cyt*68 does not code for the 19-kd tumor antigen but codes for the normal

53-kd tumor antigen in infected KB cells (manuscript in preparation). The *cyt* phenotype caused by various point mutations may be due to alterations in specific domains of the 19-kd tumor antigen.

Cell transformation. To determine the effect of the *dl*250 mutation on cell transformation an established REF cell line, CREF (7), and two kinds of primary cells, REF and BRK, were infected with Ad2 *wt* or *dl*250 and assayed for the formation of transformed foci. These results are presented in Table 1. Mutant *dl*250 transformed the rat embryo cells (CREF and REF) at a greatly reduced frequency (5- to 12-fold) compared with Ad2 *wt*. In addition, the foci induced by the mutant were generally flat. It is rather difficult to assess the efficiency of transformation on our BRK cells. These cells are transformed by Ad2 *wt* over a very narrow range of multiplicity of infection. Higher multiplicities of infection by Ad2 *wt* do not result in transformed foci due to killing of cells. In the experiment shown in Table 1, Ad2 *wt* yielded 20 foci at a multiplicity of 1.5 virus particles per cell, whereas mutant *dl*250 did not transform. Mutant *dl*250 also did not yield transformed foci at a multiplicity of 6 virus particles per cell. However, at a multiplicity of 12 virus particles per cell, mutant *dl*250 yielded 2.5 foci per dish. By extrapolation, we estimate that the efficiency of transformation by *dl*250 is only ca. 2% that of Ad2 *wt*.

We subcultured the transformed cells from individual foci formed on CREF cells and assayed them for anchorage-independent growth on soft agar. Cells transformed by Ad2 *wt* from two different foci readily formed macroscopic colonies within 2 weeks, whereas the cells (from three different foci) transformed by *dl*250 formed very small colonies, which were slightly larger than the colonies formed by the spontaneously transformed cells (Fig. 7). This indicates that the cells transformed by *dl*250 are deficient in anchorage-independent growth.

DISCUSSION

We constructed a viable deletion mutant of Ad2 (*dl*250) lacking most of the 19-kd tumor antigen-coding region of early region E1b. Mutant *dl*250 produces large clear plaques on human cells and is cytocidal on infected cells. Recently, viable Ad12 mutants which have either deletions or insertions within the 19-kd tumor antigen-coding region have been isolated (9). It is not known whether these mutants produce large plaques or the *cyt* phenotype. However, both in Ad2 and Ad12, the 19-kd tumor antigen appears not to be fully essential for viral replication. In the absence of functional 19-kd tumor antigen, the yield of progeny virus is reduced ca. 100-fold in KB cells infected with *dl*250 (Fig. 4). We recently showed that mutant *dl*250 is defective in effi-

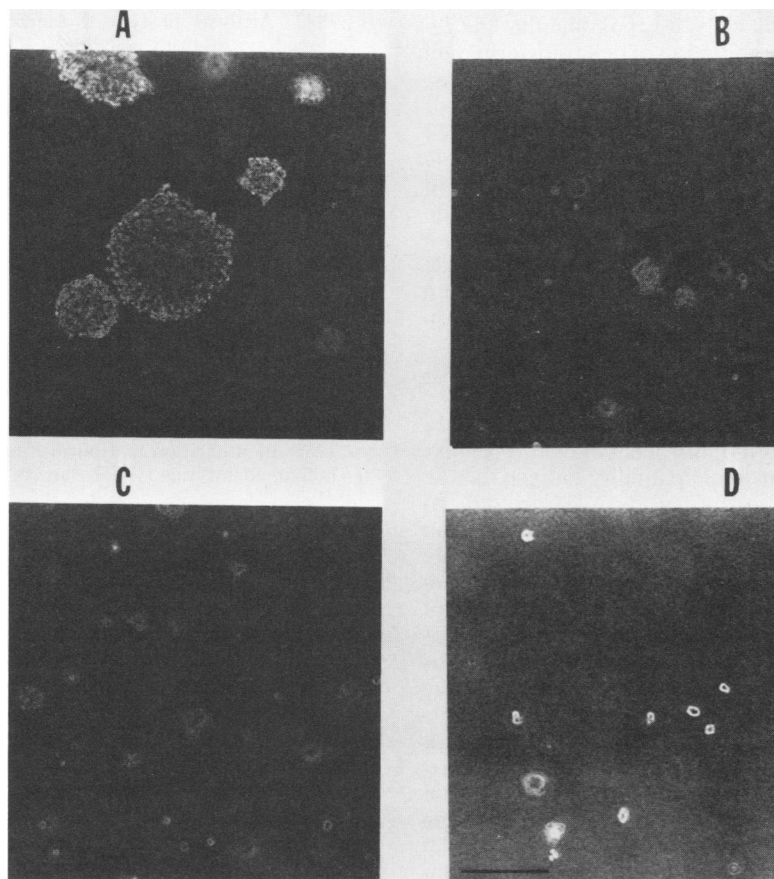


FIG. 7. Colonies of transformed CREF cells on soft agar. Anchorage-independent growth of different cell lines were determined by assaying colony formation in 0.3% agarose-containing growth medium over an underlay of 0.5% agarose-containing growth medium. Photos of living colonies were taken on a Nikon inverted stage microscope. (A) *wt*-transformed cell line; (B and C) two different *dl*250-transformed cell lines; (D) spontaneously transformed cell lines. Bar, 0.2 mm.

cient viral DNA synthesis and in protection of newly replicated DNA (T. Subramanian, M. Kuppuswamy, J. Gysbers, S. Mak, and G. Chinnadurai, J. Biol. Chem., in press). The reduced viral multiplication may therefore be due to defective viral DNA replication. However, other Ad2 *lp* mutants which produce altered 19-kd tumor antigen produce *wt* levels of progeny virus. It is interesting to note that mutant *lp*5 produces much reduced amounts of the altered 19-kd tumor antigen (5), but the mutation does not affect the virus growth. It is possible that this protein is required in small amounts for efficient viral multiplication.

Although the 19-kd tumor antigen is not an absolute requirement for viral multiplication, it appears to be important for cell transformation. Mutant *dl*250 transforms established rat embryo cells and primary REF and BRK cells at greatly reduced frequency. The defect of *dl*250 in cell transformation is not due to any other defect in the synthesis of other E1 products. In cells infected with *dl*250, accumulation of E1a and E1b mRNAs are normal (data not shown). Like *dl*250, Ad12 *cyt* mutants (18, 23), Ad12 mutants with lesions in the 19-kd tumor antigen-coding region (9), and Ad2 *lp* mutants that have point mutations within the 19-kd tumor antigen-coding region (5) are transformation defective. Apart from greatly reduced transformation frequency, cells transformed by *dl*250 are defective in anchorage-independent growth. These results demonstrate that the presence of the E1b 19-kd tumor antigen is important for oncogenic transformation of rat cells in conjunction with E1a tumor antigens. Since transformed foci (at greatly reduced frequency) were observed in *dl*250-infected rat cells, it is possible that the 19-kd protein is not required for initiation of cell transformation but may be involved in the maintenance of the fully transformed state.

We mapped the *cyt* mutants of highly oncogenic Ad12 within the 19-kd tumor antigen-coding region by intertypic complementation analysis. The Ad12 *cyt* mutants are also transformation defective (18, 23) and are nononcogenic in newborn hamsters (23). Earlier genetic studies have shown that the *cyt* mutants we examined have mutations in a single locus (24). It is therefore clear that the 19-kd tumor antigen plays a crucial role directly or indirectly in tumorigenesis, in addition to in vitro cell transformation.

Recently a novel mechanism for tumorigenesis of Ad12-transformed cells in newborm syngeneic rats was postulated (3, 22). One of the two tumor antigens coded by the E1a region (coded by a 13S mRNA) has been shown to repress the expression of a major histocompatibility antigen. These findings suggest that cells transformed by Ad12 are tumorigenic in immunocompetent syngeneic rats by escaping from the T-cell-mediated immune response. Our results indicate that the E1b-coded 19-kd tumor antigen that is defective in Ad12 *cyt* mutants may be important for tumorigenesis of newborn hamsters. It is therefore possible that the mechanism of tumorigenesis in hamsters and rats is different, or that the role of the 19-kd tumor antigen in tumorigenesis may be indirect. The 19-kd tumor antigen resembles other transforming proteins, such as polyoma middle T-antigen (15), $pp60^{src}$ (17, 27), and Ha-ras (28) in that it is a membrane-associated protein. Unfortunately not much is known about the biochemical functions of this adenovirus-transforming protein. Detailed functional characterization of the 19-kd tumor antigen should illuminate its role in cell transformation and in tumorigenesis.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Anderson, C. W., and J. B. Lewis.** 1980. Aminoterminal sequence of adenovirus type 2 proteins: hexon, fiber, component IV, and early protein 1B-15K. Virology **104**:27–41.
2. **Anderson, C. W., R. C. Schmitt, J. E. Smart, and J. B. Lewis.** 1984. Early region 1B of adenovirus 2 encodes two coterminal proteins of 495 and 155 amino acid residues. J. Virol. **50**:387–396.
3. **Bernards, R., P. I. Schrier, A. Houweling, J. L. Bos, and A. J. van der Eb.** 1983. Tumorigenicity of cells transformed by adenovirus type 12 by evasion of T-cell immunity. Nature (London) **305**:776–779.
4. **Brusca, J. S., and G. Chinnadurai.** 1981. Transforming genes among three different oncogenic subgroups of human adenoviruses have similar replicative functions. J. Virol. **39**:300–305.
5. **Chinnadurai, G.** 1983. Adenovirus 2 lp^+ locus codes for a 19-kd tumor antigen that plays an essential role in cell transformation. Cell **33**:759–766.
6. **Chinnadurai, G., S. Chinnadurai, and J. Brusca.** 1979. Physical mapping of a large-plaque mutation of adenovirus type 2. J. Virol. **32**:623–628.
7. **Fisher, P. B., L. E. Babiss, I. B. Weinstein, and H. S. Ginsberg.** 1982. Analysis of type 5 adenovirus transformation with a cloned rat embryo cell line (CREF). Proc. Natl. Acad. Sci. U.S.A. **79**:3527–3531.
8. **Flint, S. J., and T. R. Broker.** 1980. Structure and genomic organization of adenoviruses, p. 383–442. *In* J. Tooze (ed.), DNA tumor viruses, molecular biology of tumor viruses, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **Fukui, Y., I. Saito, K. Shiroki, and H. Shimojo.** 1984. Isolation of transformation-defective, replication-nondefective early region 1B mutants of adenovirus 12. J. Virol. **49**:154–161.
10. **Gaynor, R. B., A. Tsukamoto, C. Montell, and A. J. Berk.** 1982. Enhanced expression of adenovirus transforming proteins. J. Virol. **44**:276–285.
11. **Gingeras, T. R., D. Sciaky, R. E. Gelinas, J. Bing-Dong, C. Yen, M. Kelly, P. Bullock, B. Parson, K. O'Neil, and R. J. Roberts.** 1982. Nucleotide sequences from the adenovirus-2 genome. J. Biol. Chem. **257**:13576–13491.
12. **Green, M., K. H. Brackmann, M. A. Cartas, and T. Matsuo.** 1982. Identification and purification of a protein coded by the human adenovirus type 2 transforming region. J. Virol. **42**:30–41.
13. **Green, M., K. H. Brackmann, L. A. Lucher, J. S. Symington, and T. A. Kramer.** 1983. Human adenovirus 2 E1b-19K and E1b-53K tumor antigens: antipeptide antibodies targeted to the NH_2 and COOH termini. J. Virol. **48**:604–615.
14. **Green, M., W. S. M. Wold, K. H. Brackmann, and M. A. Cartas.** 1979. Identification of families of overlapping polypeptides coded by early "transforming" gene region 1 of human adenovirus type 2. Virology **97**:275–286.
15. **Ito, Y.** 1979. Polyoma virus-specific 55K protein isolated from plasma membrane of productively infected cells is virus-coded and important for cell transformation. Virology **98**:261–266.
16. **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.
17. **Levinson, A. D., S. A. Courtneidge, and J. M. Bishop.** 1980. Structural and functional domains of the Rous sarcoma virus transforming protein ($pp60^{src}$). Proc. Natl. Acad. Sci. U.S.A. **78**:1624–1628.
18. **Mak, I., and S. Mak.** 1983. Transformation of rat cells by *cyt* mutants of adenovirus type 12 and mutants of adenovirus type

5. J. Virol. **45**:1107–1117.
19. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Maxam, A., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base specific chemical cleavages. Methods Enzymol. **65**:499–560.
21. **Rowe, D. T., and F. L. Graham.** 1981. Complementation of adenovirus type 5 host range mutants by adenovirus type 12 in coinfected HeLa and BHK-21 cells. J. Virol. **38**:191–197.
22. **Schrier, P. I., R. Bernards, R. T. M. J. Vaessen, A. Houweling, and A. J. van der Eb.** 1983. Expression of class I major histocompatibility antigens switched off by highly oncogenic adenovirus 12 in transformed rat cells. Nature (London) **305**:771–775.
23. **Takemori, N., J. L. Riggs, and C. D. Aldrich.** 1968. Genetic studies with tumorigenic adenovirus 1. Isolation of cytocidal (*cyt*) mutants of adenovirus type 12. Virology **36**:575–586.
24. **Takemori, N., J. L. Riggs, and C. D. Aldrich.** 1969. Genetic studies with tumorigenic adenoviruses. II. Heterogeneity of *cyt* mutants of adenovirus type 12. Virology **38**:8–15.
25. **Virtanen, A., and U. Pettersson.** 1983. The molecular structure of the 9S mRNA from early region 1A of adenovirus serotype 2. J. Mol. Biol. **165**:496–499.
26. **Williams, J., U. Ho, and R. Galos.** 1981. Evidence for functional relatedness of products encoded by the transforming sequences of human adenovirus types 5 and 12. Virology **110**:208–212.
27. **Willingham, M. C., G. Jay, and I. Pastan.** 1979. Localization of the avian sarcoma virus *src* gene product to the plasma membrane of transformed cells by electron microscopic immunocytochemistry. Cell **18**:125–134.
28. **Willingham, M. C., I. Pastan, T. Y. Shih, and E. M. Scolnick.** 1980. Localization of the *src* gene product of Harvey strain of MSV to plasma membrane of transformed cells by electron microscopic immunocytochemistry. Cell **19**:1005–1014.
29. **Wold, W. S. M., and M. Green.** 1979. Adenovirus type 2 early polypeptides immunoprecipitated by antisera to five lines of adenovirus-transformed rat cells. J. Virol. **30**:297–310.