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Deletion of the Gene Encoding the Adenovirus 5 Early Region 1B 21,000-Molecular-Weight Polypeptide Leads to Degradation of Viral and Host Cell DNA

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The adenovirus 5 mutant H5*dl*337 lacks 146 base pairs within early region 1B. The deletion removes a portion of the region encoding the E1B 21,000-molecular-weight (21K) polypeptide, but does not disturb the E1B-55K/17K coding region. The virus is slightly defective for growth in cultured HeLa cells, in which its final yield is reduced ca. 10-fold compared with wild-type virus. The mutant displays a striking phenotype in HeLa cells. The onset of cytopathic effect is dramatically accelerated, and both host cell and viral DNAs are extensively degraded late after infection. This defect has been described previously for a variety of adenovirus mutants and has been termed a cytocidal (*cyt*) phenotype. H5*dl*337 serves to map this defect to the loss of E1B-21K polypeptide function. In addition to its defect in the productive growth cycle, H5*dl*337 is unable to transform rat cells at normal efficiency.

The adenovirus (Ad) early region 1B (E1B) transcription unit is located between ca. 4.5 and 11.5 map units on the viral chromosome. The region provides functions required for both oncogenic transformation and productive growth in cultured cells (3, 7, 12, 15, 19, 20, 25, 28, 30). It also encodes one or more functions responsible for the greater tumorigenicity of subgroup A as compared with subgroup C Ad in nude mice (4).

The E1B transcription unit encodes three mRNAs early after infection. The largest mRNA (22S) can code both 21,000- and 55,000-molecular-weight (21K and 55K) polypeptides, whereas the smallest (13S) codes only the 21K species (6). The 55K coding region overlaps the C-terminal 40% of the 21K region in a second reading frame. Recently, Anderson et al. (2) have identified an additional E1B-coded polypeptide (17K) synthesized from an intermediate-sized mRNA. The 17K moiety is encoded in the 55K reading frame and comprises the amino- and carboxy-terminal segment of the larger protein. The 17K polypeptide is probably the E1B product previously reported as an 18K (9) or 20K species (22). The synthesis of a fourth mRNA (9S) is directed by a control element within the E1B region which becomes active late after infection. This small, unspliced mRNA encodes a 14K structural polypeptide (IX). All of the RNAs discussed so far are encoded by the same DNA strand. There are also several small open reading frames on the opposite DNA strand (17) that may be utilized (32).

To assign specific functions to individual polypeptides, we have constructed several viruses carrying small deletion mutations in the E1B region. Here we report the initial characterization of H5*dl*337, which lacks 146 base pairs within the E1B region. The mutation removes a portion of the coding region for the E1B-21K polypeptide, but does not disturb the 55K or 17K polypeptides and also does not impinge on the open reading frames located on the second DNA strand in the E1B region. The mutant is defective for transformation of rat cells and displays a striking phenotype in productively infected HeLa cells. The onset of cytopathic effect is dramatically accelerated, and both host cell and viral DNAs are extensively degraded late after infection.

A number of Ad mutants with this phenotype have been described previously. The Ad12 cytocidal (*cyt*) mutants isolated by Takemori et al. (48, 49) display a more rapid onset and extensive cytopathic effect than that observed with wild-type Ad12. Ezoe et al. (10) demonstrated that these mutants degrade cell DNA, and Lai Fatt and Mak (33) mapped the Ad12 *cyt* mutations to early region 1B and showed that the H5*dl*313 E1B mutant was also a *cyt* variant. Recently, Stillman et al. (47) have segregated a nonconditional *cyt* mutation out of a temperature-sensitive Ad2 variant (H2*ts*111) and mapped the mutation to the region between 3.8 and 9.1 map units.

The *dl*337 phenotype, then, is entirely consistent with previous observations and definitively maps the *cyt* phenotype to the E1B-21K polypeptide.

MATERIALS AND METHODS

Viruses and cells. H5*dl*309 served as the wild-type Ad5 parent in this study. This virus was generated from H5*wt*300, which is a plaque-purified derivative of a virus stock obtained from H. Ginsberg, Columbia University, New York. H5*dl*309 was selected as a variant that contains only one *Xba*I cleavage site, located at 3.8 map units (1,339 base pairs from the left end of the chromosome), and it displays a wild-type phenotype (30). H5*dl*337 carries a 146-base-pair deletion in the E1B coding region located between nucleotide sequence positions 1770 and 1916. Its deletion was originally constructed in a recombinant plasmid, pA5*Xho*I-C (26), by dropping the Ad5 segment bordered by the *Sac*I and *BstE*II restriction endonuclease cleavage sites at Ad sequence positions 1770 and 1915, respectively. The resulting deletion junction was determined by nucleotide sequence analysis (35). The mutated Ad5 segment was rebuilt into an intact viral chromosome by overlap recombination (8, 28). H5*dl*309 donated its 3.8-to-100-map unit, *Xba*I-generated fragment to the recombination reaction. All virus infections were at a multiplicity of 20 PFU per cell unless otherwise noted in figure legends.

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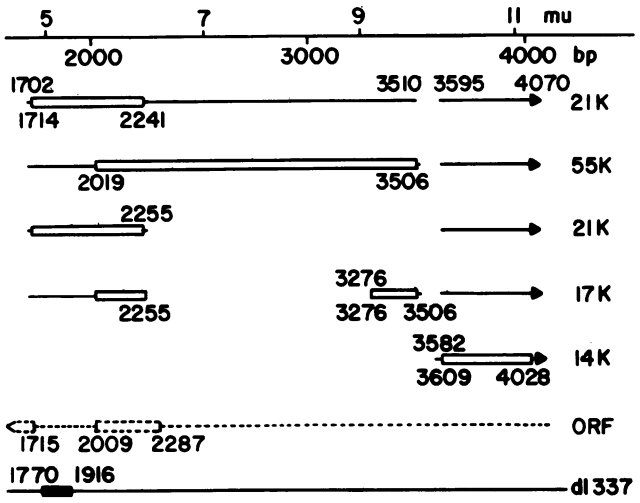

FIG. 1. Physical map of E1B mRNAs, coding regions, and the *dl*337 mutation. The top of the figure positions the map in terms of map units and nucleotide sequence position relative to the left end of the viral chromosome (6). mRNAs are indicated by lines, introns are indicated by spaces, and coding regions are indicated by open rectangles. The numbers above the lines correspond to exon end points, and the numbers below the lines correspond to coding regions. Open reading frames (ORFs) encoded by the opposite DNA strand are indicated by open rectangles outlined with a broken line. The segment deleted in *dl*337 is represented by a solid rectangle. The nucleotide sequence numbers of the base pairs present bracketing the deletion are indicated.

The 293 cell line (a human embryonic kidney cell line transformed with a DNA fragment carrying the left 11% of the Ad5 genome) was obtained from H. Young, Columbia University, New York, N.Y., and has been described by Graham et al. (21). Cells were maintained in medium containing 10% calf serum. Spinner culture HeLa cells were obtained from the American Type Culture Collection, Rockville, Md., and grown in medium supplemented with 7.5% calf serum. Primary rat embryo cells were prepared from 15-day-old Fisher rat embryos and propagated in medium containing 10% calf serum, and cloned rat embryo fibroblast cells, an established rat embryo cell line (11), were obtained from P. Fisher, Columbia University and maintained in medium supplemented with 7.5% fetal calf serum.

Preparation and analysis of DNA. Viral and cellular DNA replication rates were assayed by labeling (30 min) HeLa cell cultures with [^{3}H]thymidine (100 μCi/ml, 50 Ci/mmol) at various times after infection. Total cellular DNA was prepared and analyzed by equilibrium density centrifugation. Gradients were fractionated and radioactivity was measured.

Two procedures were used to prepare DNA for monitoring degradation. The first approach was to isolate nuclei from infected cells (31) and then to prepare high-molecular-weight DNA (5) for analysis. The second approach was to isolate low-molecular-weight DNA by the procedure of Hirt (27). The DNA was then extracted once with phenol and twice with chloroform-isoamyl alcohol, 24:1 (vol/vol), and then precipitated with ethanol. The DNA was suspended and digested sequentially with pancreatic RNase (100 μg/ml) and pronase (200 μg/ml) in the presence of sodium dodecyl sulfate (SDS) (0.5%) for 1 h each at 37°C. DNA was re-extracted with phenol and chloroform-isoamyl alcohol and was precipitated with ethanol. The DNA was then resuspended in sample buffer for electrophoretic analysis.

Southern-type analysis was essentially as described by Mougneau et al. (37), with probe DNAs labeled with ^{32}P by nick translation (42).

DNA was prepared from purified virions enriched for particles less dense than normal, essentially as described by Tibbetts (50). Briefly, virions were released from HeLa cells at 48 h after infection by sonication and banded to equilibrium in a cesium chloride-containing gradient. The particles banding at the less dense side of the virion peak (ca. 20% of the total) were subjected to a second round of equilibrium density centrifugation. This gradient was fractionated, and virion DNA was extracted (29) for analysis.

Polypeptide analysis. One hour before being labeled, infected HeLa cells were placed in medium lacking methionine and supplemented with 2% calf serum. Cultures were labeled with [^{35}S]methionine (50 μCi/ml, 1,100 Ci/mmol) for 30 min unless otherwise noted. Preparation of cellular extracts, immunoprecipitations, and SDS-polyacrylamide gel electrophoresis were carried out as described by Sarnow et al. (43). The E1B-55K-specific monoclonal antibody was the gift of A. Levine, Princeton University, Princeton, N.J., and has been described previously (44). The E1B-21K-specific antiserum was the gift of K. Brackmann and M. Green, St. Louis University, St. Louis, Mo. It was prepared by immunization with a synthetic peptide corresponding to the N-terminal region of the 21K species (23).

Transformation experiments. Transformation experiments utilized either the established cloned rat embryo fibroblast cell line or primary rat embryo cells. Cells were infected at ca. 80% confluence with virions that had been purified by equilibrium density centrifugation and suspended in Tris-saline plus 2% calf serum. Adsorption was for 1 h at 37°C and was followed by two washes using medium without serum. Medium containing 10% calf serum was added, and incubation was continued for 1 day at 37°C. The cultures were then passaged (1:5), and after three additional days of incubation in standard medium they were placed in medium containing 0.1 mM $CaCl_2$ (13). Cells were refed with this low-calcium medium twice a week, and final counts of foci were made at 6 weeks.

RESULTS

Construction and propagation of H5*dl*337. The 337 mutation was initially constructed in a recombinant plasmid

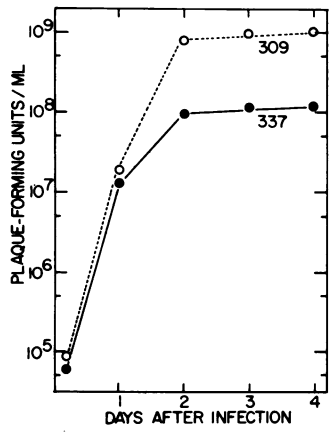

FIG. 2. Growth kinetics of mutant and parental viruses in HeLa cells. Cells were infected at a multiplicity of 3 PFU per cell, and the virus yield was assayed at the times indicated by plaque assay on 293 cells. Symbols: ●, *dl*337; ○, *dl*309.

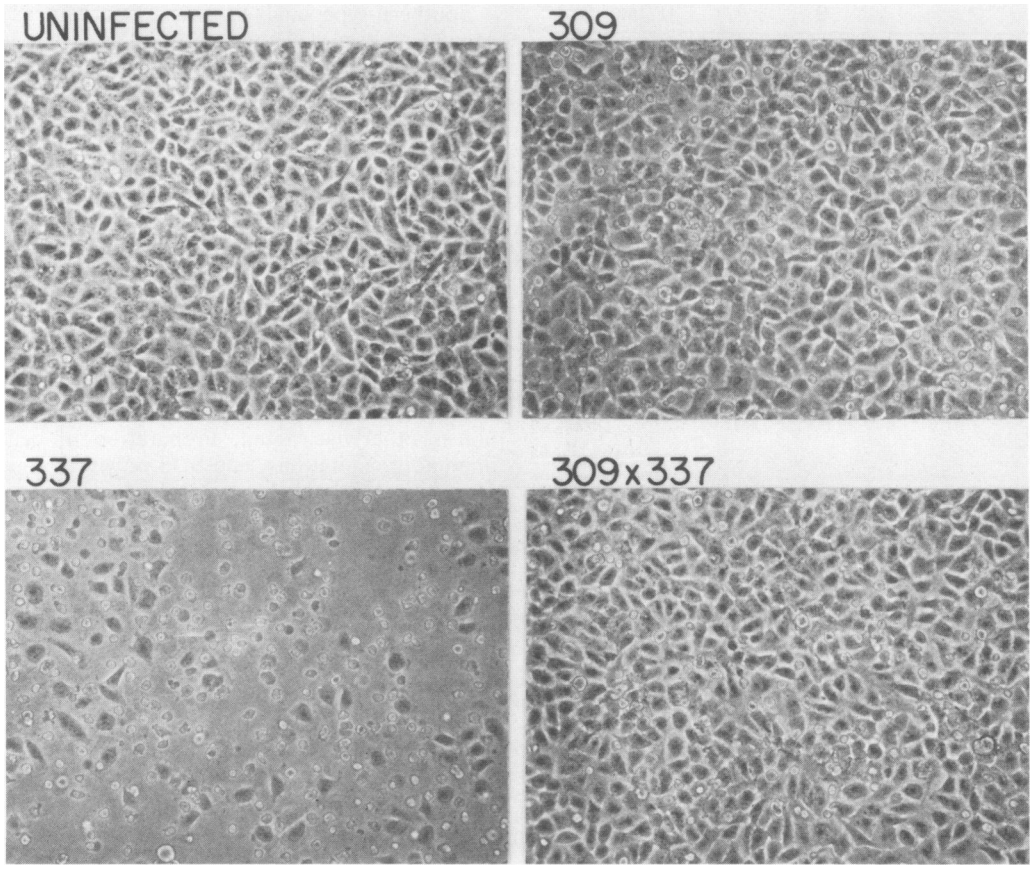

FIG. 3. Photographs of HeLa cell cultures infected with wild-type or mutant viruses or both. At 36 h after infection at a multiplicity of 20 PFU per cell, cultures were photographed at a 40× magnification.

carrying the left-end 5,580 base pairs (0 to 15.5 map units) of the Ad5 chromosome. A DNA segment bordered by *Sac*I (sequence position 1770) and *Bst*EII (position 1915) cleavage sites was excised. The mutated segment was then rebuilt into a viral chromosome employing overlap recombination (8, 28). In this procedure, two overlapping DNAs are used to transfect cells, neither of which represents an intact viral genome. Recombination occurs within the transfected cells

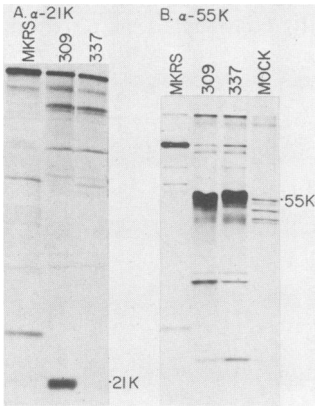

FIG. 4. Analysis of E1B-specific polypeptides produced in mutant and wild-type virus-infected HeLa cells. Cells were labeled for 60 min with [^{35}S]methionine at 24 h after infection. Extracts were prepared, and immunoprecipitations were carried out with either E1B-21K-specific (A) or E1B-55K-specific (B) antibodies. Electrophoresis was in a 12.5% polyacrylamide gel containing SDS. Bands representing the E1B-21K and -55K moieties are indicated beside the autoradiograms.

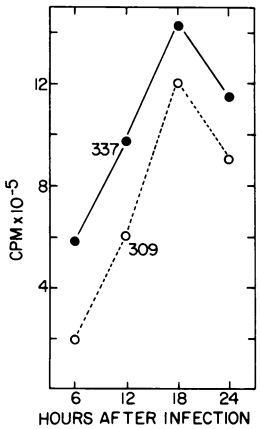

FIG. 5. Determination of the rates of viral DNA synthesis in mutant and wild-type virus-infected HeLa cells. Cells were labeled for 30 min with [^{3}H]thymidine at various times after infection. Total cellular DNA was prepared, and DNAs of viral and host cell origin were separated by equilibrium density centrifugation. The total radioactivity in the viral species was determined, and the results are plotted as a function of time. Symbols: ●, *dl*337; ○, *dl*309.

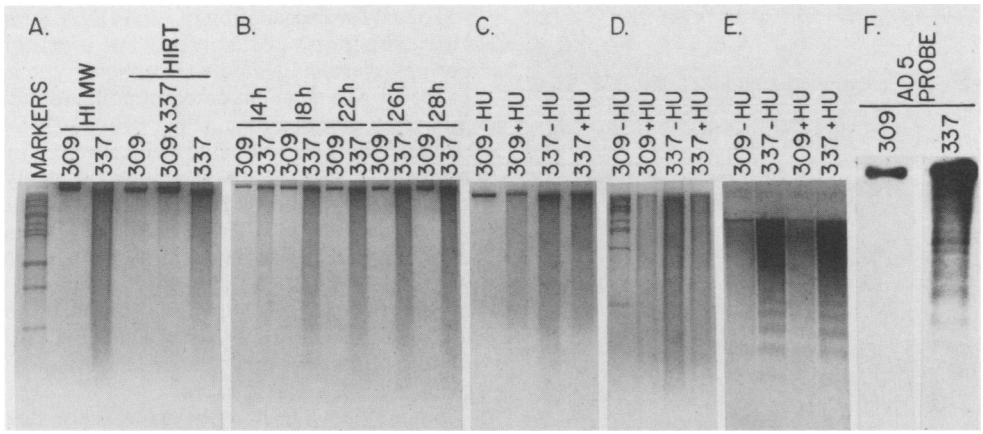

FIG. 6. Analysis of host cell and viral DNAs after infection of HeLa cells with mutant and wild-type viruses. DNA was prepared at various times after infection, subjected to electrophoresis in 1.0% agarose gels in the presence of ethidium bromide, and visualized by fluorescence. In the case of high-molecular-weight DNA, equal amounts of DNA were loaded onto gels; for DNA extracted by the procedure of Hirt, equal volumes of extract were subjected to electrophoresis. (A) Comparison of DNAs isolated at 24 h after infection of HeLa Spinner cells by means of an extraction procedure designed to yield high-molecular-weight DNA (5) or by Hirt (27) extraction. The heading 337 × 309 indicates cells coinfected with *dl*337 and *dl*309 at equal multiplicities. The marker is a *Sma*I endonuclease digestion of *dl*337 DNA. (B) Comparison of DNAs extracted from infected cells by the Hirt procedure at various times after infection. (C) Comparison of DNAs extracted by the Hirt procedure at 26 h after infection (20 PFU per cell) and maintenance of cultures in the presence or absence of hydroxyurea (10 mM). (D) *Hin*dIII endonuclease digest of the DNAs displayed in C. (E) Identical to the experiment described in C, except that infection was at a multiplicity of 100 PFU per cell. (F) Southern-type analysis of DNAs extracted at 24 h after infection by the Hirt procedure. The probe DNA was a recombinant plasmid carrying the 85-to-100-map unit region of the Ad5 chromosome. The corresponding total DNA patterns are those shown in A.

in the overlap region to generate an intact and infectious chromosome. The fragments utilized in this reconstruction contained the 0-to-15.5- and 3.8-to-100-map unit domains of the Ad5 chromosome, providing an opportunity for recombination between the 3.8 and 15.5 map unit positions. Recombination events occurring between the deletion endpoint at ca. 5.5 and 15.5 map units fixed the 337 mutation into the viral chromosome.

The deletion was precisely localized by DNA sequence analysis. *dl*337 lacks 146 base pairs between sequence positions 1770 and 1916 (Fig. 1). It removes sequences encoding the E1B-21K polypeptide, but does not alter either the 55K or 17K species or the open reading frames present on the opposite strand. The deletion alters the 21K reading frame, generating a stop signal 29 codons beyond the deletion endpoint. In theory, a 47-amino acid derivative of the 21K polypeptide could be produced consisting of 19 residues from the 21K amino terminus appended to 28 novel amino acids.

The variant was propagated in 293 cells whose resident E1 coding region (1, 21) should complement a defect arising from the 337 mutation. The severity of the growth defect of the mutant was evaluated by comparing its growth in HeLa cells with that of its wild-type parent, *dl*309 (Fig. 2). The rate of virus production in *dl*337-infected cells appeared normal, but its final yield was reduced ca. 10-fold compared with *dl*309. In addition to growth capability, this experiment revealed a striking characteristic of *dl*337 infection. The mutant is considerably more cytopathic than its parent. This characteristic is documented in Fig. 3. By 36 h after infection at a multiplicity of 20 PFU per cell, ca. 80% of the cells in a mutant-infected culture were stripped off the surface of the culture dish, while the pathological effects of virus replication were just becoming evident in the wild-type virus-infected culture. The increased cytopathic effect proved to be recessive in mixedly infected cultures (Fig. 3). As discussed above, increased cytopathogenicity has been noted previously for Ad12, Ad5, and Ad2 mutants. *dl*337 unambiguously maps a locus in which a mutation can induce the *cyt* phenotype.

Production of E1B-specific polypeptides by H5*dl*337. The alteration in *dl*337 should prevent synthesis of the E1B-21K polypeptide, but not of the 55K species. To confirm that this was indeed the case, HeLa cells were labeled with [^{35}S]methionine for 60 min at 24 h after infection with mutant or wild-type viruses, and the synthesis of E1B-coded polypeptides was monitored by immunoprecipitation with specific antibodies. No 21K polypeptide was detected in *dl*337-infected cells (Fig. 4A), and synthesis of the 55K moiety was not altered (Fig. 4B).

DNA replication appears normal in H5*dl*337-infected cells. Viral DNA replication was measured by labeling cultures with [^{3}H]thymidine for short periods at various times after infection, extracting total DNA, and separating the more dense viral segments from less dense cellular segments by equilibrium density centrifugation. The amount of radioactivity in the viral fraction was then quantitated (Fig. 5). We observed a slight but reproducible enhancement in the rate of mutant versus wild-type virus replication at each time point tested. Very little difference in accumulation of DNA was evident when DNAs were monitored by ethidium bromide intercalation and fluorescence until ca. 20 h after infection (data not shown). This makes sense because the difference in thymidine incorporation was considerably less than twofold at most times tested. Further, part of the incorporation of labeled nucleotide could result from repair synthesis at nicks in *dl*337-infected cells in which DNA is degraded.

H5*dl*337-infected cells exhibit extensive DNA degradation. Degradation of DNA was readily apparent after infection of HeLa cells with *dl*337 when either high-molecular-weight DNA or DNA isolated by the Hirt procedure was analyzed by agarose gel electrophoresis (Fig. 6A). DNA from *dl*337-infected cells showed extensive smearing of DNA fragments

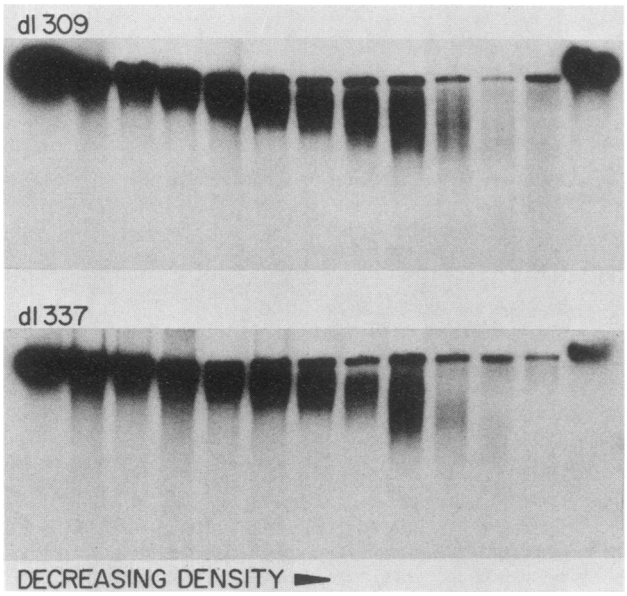

FIG. 7. Analysis of DNAs present in mutant and wild-type virions that were produced in HeLa cells and enriched for less dense particles than normal. Virions were released from infected cells at 48 h after infection and banded to equilibrium in a cesium chloride-containing gradient. Particles banding at the less dense side of the virion peak were subjected to a second round of equilibrium density centrifugation. This gradient was fractionated and virion DNA was extracted. DNAs were subjected to electrophoresis in 0.5% agarose gels in the presence of ethidium bromide and were visualized by fluorescence. The first and last samples of each gradient are markers of uncut viral DNA. Size markers were included in the gel and stained with ethidium bromide. The degraded DNA extends down to ca. 30% of intact viral DNA length.

through the region of the gel where lower-molecular-weight species migrate. A mixed infection utilizing equal input multiplicities of mutant and wild-type viruses did not result in DNA degradation, indicating that the phenotype was recessive (Fig. 6A). This is consistent with the recessive character of the *cyt* phenotype demonstrated in Fig. 3. Examination of low-molecular-weight DNA from HeLa spinner cell cultures revealed that degradation was apparent at the earliest time assayed (14 h) and reached its maximum at ca. 22 h after infection (Fig. 6B).

So far, our experiments have not reliably distinguished whether viral DNA, cell DNA, or both are degraded. To test for degradation of cell DNA, HeLa cultures were infected and then fed with medium containing hydroxyurea to prevent DNA replication. DNA degradation was readily apparent in *dl*337-infected cultures. Unexpectedly, *dl*309-infected cells showed evidence of DNA degradation, although to a lesser extent (Fig. 6C). The difference between mutant and wild type became much greater in cells infected at a very high input multiplicity (Fig. 6E). Since viral DNA had not replicated in the presence of hydroxyurea (Fig. 6D), the degraded DNA must have been cellular in origin. Further, since hydroxyurea prevented *dl*337 from entering the late phase of gene expression, we can conclude that an early viral gene product either is the nuclease itself or induces a cellular nuclease. The hydroxyurea experiment is consistent with the earlier data of Lai Fatt and Mak (33), who demonstrated that infection with H5*dl*313, which is defective for DNA replication (30), nevertheless displays a degradation phenotype.

To test for degradation of viral DNA, samples prepared by Hirt extraction of cells infected at a multiplicity of 20 PFU per cell were subjected to Southern-type analysis, using as probe an Ad5 segment corresponding to the right-end 15% of the viral chromosome (Fig. 6F). Ad5 DNA was clearly degraded in *dl*337-infected cells. Interestingly, the blot hybridization revealed a series of relatively discrete bands rather than a smear. Possibly, the nuclease preferentially cleaves at specific sites dictated by the structure of the viral chromatin. No specific pattern of degradation was discernible for total DNA late after infection with *dl*337 at a multiplicity of 20 PFU per cell (Fig. 6B); however, at higher multiplicities (100 PFU per cell; Fig. 6E) a degradation pattern suggestive of a cellular DNA nucleosomal ladder was evident.

Since viral DNA was extensively degraded in *dl*337-infected cells, we checked for encapsidation of viral DNA fragments. Virions were prepared from *dl*337- or *dl*309-infected cells and banded to equilibrium in a cesium chloride-containing gradient. The particles banding at the less dense side of the peak (ca. 20% of the total) were subjected to a second round of equilibrium density centrifugation. This gradient was fractionated, and virion DNA was extracted and analyzed by electrophoresis (Fig. 7). Shortened DNAs were evident in fractions containing less-dense virions. This

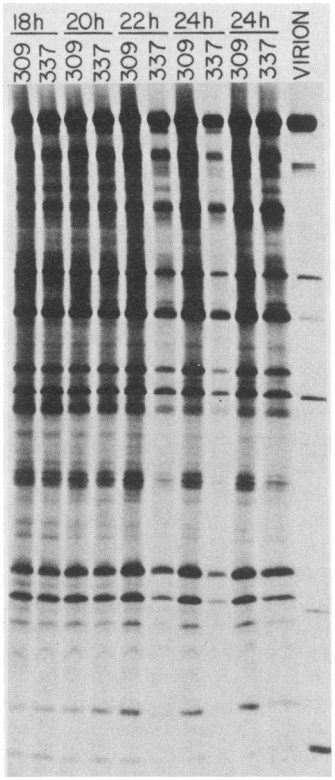

FIG. 8. Analysis of polypeptides synthesized in HeLa cells infected with mutant and wild-type viruses. Cultures were labeled for 30 min with [^{35}S]methionine at the times indicated after infection. Cell extracts were prepared, and samples were subjected to electrophoresis in a 12.5% polyacrylamide gel containing SDS. Equal amounts of total protein were added to the first eight lanes of the gel, and equal amounts of radioactivity were added to the second set of 24-h samples.

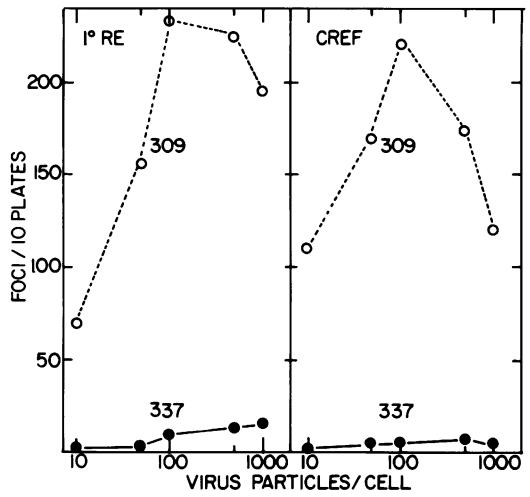

FIG. 9. Transformation of rat cells with mutant or wild-type viruses. Transformations were carried out by using equilibrium density gradient-purified virions to infect either primary rat embryo cells or the established cloned rat embryo fibroblast cell line. Foci were counted 6 weeks after infection. Symbols: ○, *dl*337; ●, *dl*309.

phenomenon appears similar to that described by Tibbetts (50) and Hammarskjold and Winberg (24) for subgroup B adenoviruses, which produce large amounts of particles less dense than wild-type virions. However, in the context of our present experiment, there was no difference between mutant and wild-type virus-infected cells. Apparently, the viral DNA fragments in *dl*337-infected cells are excluded from virions.

Protein synthesis is reduced late after infection with H5*dl*337. The synthesis of late polypeptides was monitored by labeling HeLa cells with [^{35}S]methionine for brief periods at various times after infection, extracting total cell protein, and subjecting samples to electrophoresis in an SDS-containing polyacrylamide gel (Fig. 8). The mutant produced the same variety of polypeptides in the same relative amounts as did the wild-type virus. However, by 22 h after infection, the level of late polypeptide synthesis was reduced (ca. fourfold) in *dl*337 as compared with *dl*309-infected cells. Presumably, this reduction was an indirect effect of the mutation, resulting from the increased cytopathogenicity and DNA degradation that occur in *dl*337-infected cultures.

H5*dl*337 is defective for transformation. As discussed above, the E1B region plays a central role in transformation by adenoviruses. Not surprisingly, *dl*337 was markedly defective for transformation of both primary rat embryo cells and the established cloned rat embryo fibroblast rat cell line (Fig. 9). In both cases, a small number of foci arose after *dl*337 infection. Two cell lines have been established from infected primary rat embryo cells. Both express the E1B-55K but not the 21K polypeptide. Neither is fully transformed because they fail to grow in agar (data not shown).

DISCUSSION

H5*dl*337 fails to synthesize the E1B-21K polypeptide (Fig. 4). The virus is severely defective for transformation of rat cells (Fig. 9), but is only moderately compromised in its ability to carry out a productive infection in HeLa cells (Fig. 2).

The mutant displays a striking phenotype during productive infection. The onset of cytopathic effect is very rapid and severe (Fig. 3), and both viral and cellular DNAs are extensively degraded (Fig. 6). A cytolytic (*cyt*) phenotype has been noted in a variety of Ad12, Ad5, and Ad2 mutants (10, 33, 47–49) and mapped to region E1B (33, 47). *dl*337 maps the *cyt* phenotype to the E1B-21K polypeptide.

We observed two molecular defects in *dl*377-infected HeLa cells. Viral and cellular DNAs were degraded (Fig. 6), and late polypeptide synthesis was reduced severalfold by 22 to 24 h as compared with a wild-type virus infection (Fig. 8). Very likely, the perturbation in late polypeptide synthesis was a secondary effect of the *cyt* phenotype and was caused by extensive DNA degradation and early cell death. Presumably, then, the 10-fold reduction in *dl*337 versus *dl*309 virus yield (Fig. 2) was due entirely to the *cyt* effect. It is not clear whether the increased cytopathogenicity of *dl*337 is a direct consequence of DNA degradation, but such a relationship appears likely.

What is the source of the nuclease? It is clearly not a mutated form of the E1B-21K polypeptide. *dl*337 could encode no more than the amino-terminal 19 amino acids of the polypeptide (Fig. 1), and its degradation phenotype was recessive (Fig. 6A). Further, *dl*313, which lacks the 21K coding region entirely (30), is also a *cyt* mutant (33). Therefore, the nuclease is either another viral gene product or a cellular product that is either induced or unmasked by adenovirus infection. In either case, the role of the E1B polypeptide must be, at least in part, to control and possibly direct the nuclease.

If the nuclease is another viral gene product, it must be an early polypeptide because (i) it is expressed in the presence of hydroxyurea, which prevents entry into the late phase of gene expression (Fig. 6), and (ii) it is expressed in cells infected with *dl*313 (33), which fails to replicate its DNA and express late polypeptides (30). *dl*313 also rules out the E1B-55K and 17K moieties as nuclease candidates since it lacks their coding region. If the nuclease is of cellular origin, it might be induced by the adenovirus E1A gene, as are certain heat shock proteins (38). The adenovirus E1B-21K gene product may be somewhat analogous to the bacteriophage lambda *gam* gene product which binds to the *Escherichia coli recBC* nuclease (exoV) and inhibits its activity (46).

If the E1B-21K polypeptide simply serves to inhibit a cellular nuclease, the putative 21K-nuclease interaction may not play any further role in the infectious cycle. However, if the nuclease is viral in origin, the E1B-21K polypeptide might serve to direct the activity of the nuclease. Since *dl*337 grows quite well in the absence of a putative 21K-nuclease interaction, it is not clear what the function of a directed nuclease might be. Conceivably, such an interaction could play a role in the high levels of recombination observed in adenovirus-infected cells (reviewed in reference 18).

Two additional features of the *dl*337 lytic phenotype deserve comment. First, the nuclease shows some specificity in the degradation of DNA (Fig. 6E and F). The ladder-type DNA cleavage raises the possibility that the structure of chromatin influences the cleavage. If the cleavage is nucleosome dependent, the E1B-21K mutation just might provide an in vivo tool with which to probe adenovirus and cellular chromatin structure. The second comment derives from the failure of degraded DNA to be packaged (Fig. 7). To be packaged, a DNA must carry the *cis*-acting packaging sequence located at the left end of the viral chromosome (24, 26, 50), and it must be an appropriate size. One might expect a substantial proportion of viral degradation products to meet these requirements, but they are not packaged. The explanation for this observation will likely await a better

understanding of both packaging and *cyt* DNA degradation mechanisms.

The *dl*337 transformation defect was not surprising. Some of the original Ad12 *cyt* mutants (34, 48, 49) as well as Ad12 mutants carrying deletion and insertion mutations in their E1B-21K coding regions (14) are defective for both transformation and tumor induction in newborn hamsters. Ad2 and Ad5 E1B-21K mutants have also been described which fail to transform. These include insertion mutations in cloned DNA fragments (36) and single-base-pair changes in mutant viruses (7).

Evidence is quite good that the 21K species functions at least in part at the cell surface. Persson et al. (39) found the polypeptide to be present in both nuclear and plasma membrane fractions of infected cells. Shiroki et al. (45) and Raska et al. (40, 41) have mapped an adenovirus tumor-specific transplantation antigen to early region 1, and Gallimore and Williams (16) have employed region E1 mutants to localize the antigen to the E1B-21K coding region. Thus, the antigen likely plays a key role at the interface between the infected or tumor cell and the host immune surveillance system. The role of the E1B-21K polypeptide in the transformation of cultured cells, whether the transforming role is carried out by the polypeptide present in the nuclear or plasma membrane compartments (or both) of the cell, and the relationship of that role, if any, to the defect observed in *dl*337-infected cells, are unclear.

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