Identification of Adenovirus 2 Early Region 4 Polypeptides by In Vitro Translation and Tryptic Peptide Map Analysis

TAKUMI MATSUO, SHUICHI HASHIMOTO, WILLIAM S. M. WOLD, JANEY SYMINGTON, ANNE RANKIN, AND MAURICE GREEN*

Institute for Molecular Virology, St. Louis University School of Medicine, St. Louis, Missouri 63110

Received 15 June 1981/Accepted 2 September 1981

The mRNA species encoded by early region 4 (E4) (map position [mp] 91.5 to 99.3) of adenovirus 2 were isolated from the polysomes of infected KB cells and were purified by hybridization to the cloned *Hind*III-F fragment (mp 89.5 to 97.3) or to *Eco*RI-C fragment (mp 89.7 to 100). The mRNA's were translated in vitro using [³⁵S]methionine as a labeled precursor in rabbit reticulocyte lysates treated with micrococcal nuclease as well as in wheat germ lysates. Five major (35,000-molecular-weight [35K], 23K, 22K, 21K, 18K) polypeptides were observed when the reticulocyte lysate was used. The 23K, 22K, 21K, and 18K polypeptides were also observed with the wheat germ lysate, as well as a very prominent 11K polypeptide; the 35K polypeptide was not observed. Assignment of these polypeptides to E4 was further established by hybrid arrested translation. Two-dimensional gel electrophoresis of a wheat germ translate resolved five polypeptides ranging from 18K to 23K, the major 11K polypeptide, and polypeptides of 10K and 9K. The in vitro 23K to 18K and 11K polypeptides migrated to approximately the same positions on two-dimensional gels as did seven 26K to 21K polypeptides and an 11K polypeptide synthesized in vivo (Brackmann et al., *J. Biol. Chem.* **255**:6772-6779, 1980). Two-dimensional tryptic peptide maps demonstrated that the 35K, 23K, 22K, 21K, and 18K polypeptides are related. The peptide map of 11K is different from those of the above polypeptides, although 11K may share one tryptic methionine polypeptide with them. These results indicate that E4 encodes a major 11K polypeptide, as well as major 35K, 23K, 22K, 21K, and 18K polypeptides.

Early region 4 (E4) is located within map position (mp) 91.5 to 99.3 on the adenovirus 2 (Ad2) genome and is transcribed from *l*-strand at early stages of infection. This region is of interest because the organization of the E4 genes appears to be very complex and because nothing is known about the functions of the E4 products in virus multiplication. E4 specifies an array of overlapping mRNA's, most or all of which are coterminal at the 5' and 3' ends, but which differ in the size and location of the intervening sequences. For example, at least six major E4 transcripts have been detected by RNA-single-stranded DNA heteroduplex studies (8, 17), three transcripts have been detected by nuclease-gel analysis (3), and as many as nine transcripts have been detected by RNA gel-transfer hybridization (11a). E4 mRNA's have heterogeneous 5' termini, and four of the major termini have been aligned with a stretch of five nucleotides at position 325 to 329 from the right-hand end of the Ad2 genome (2, 16).

Some of the polypeptide products encoded by this region have been studied. Lewis et al. (18) and Harter and Lewis (13) translated 11,000-

molecular-weight (11K), 17K, 19K, and 21K polypeptides with mRNA complementary to *Eco*RI-C fragment (mp 89.7 to 100). By immunoprecipitation and by high-resolution two-dimensional (2D) gel analysis of polypeptides labeled in vivo with [³⁵S]methionine, our laboratory has identified an 11K and seven 26K to 21K polypeptides and has provided evidence that they may be coded by E4 (6, 23). In this communication we attempt to correlate the polypeptides that we have identified in vivo (6, 23) with those that can be translated in vitro from E4-specific mRNA by using reticulocyte and wheat germ lysates.

To obtain purified early Ad2 RNA for in vitro translation, KB cells were infected with Ad2 (200 PFU/cell) for 1 h and incubated for 10 h in the presence of cycloheximide (25 µg/ml) to block the onset of viral DNA synthesis and to accumulate viral RNAs (20). From polysomes obtained from Ad2-infected or uninfected KB cells, RNA was extracted by phenol-chloroform-isoamyl alcohol and applied to a polyuridylic acid-Sepharose column (15). Polyadenylic acid-containing RNAs were hybridized to

EcoRI-C (mp 89.7 to 100), *EcoRI*-B (mp 58.5 to 70.7), and complete genome DNA, immobilized on nitrocellulose filters (7, 14, 15); the relative hybridization range was 2.5 to 4%, 0.3 to 0.4%, and 7 to 12%, respectively, in several experiments. Nine times more RNA hybridized to *EcoRI*-C than to *EcoRI*-B. Thus, E4 RNA is much more abundant than E2 RNA under these labeling conditions (15).

Figure 1A illustrates the total early Ad2- and the E4-specific polypeptides synthesized in vitro by a micrococcal nuclease-treated reticulocyte lysate (22), using the hybridization-purified

mRNA's as template, and analyzed by sodium dodecyl sulfate-polyacrylamide electrophoresis and fluorography (4). Lane A (Fig. 1A) shows the polypeptides synthesized using total late Ad2-specific mRNA. Without exogenous mRNA, globin (10K to 11K) and several larger very minor polypeptides were synthesized (lane B). When RNA was added, [³⁵S]methionine incorporated into trichloroacetic acid-precipitable material increased 5- to 20-fold, depending upon the amount of mRNA used. Lane D shows the Ad2 early polypeptides translated from mRNA selected by hybridization to the com-

FIG. 1. (A) Fluorograph of [³⁵S]methionine-labeled polypeptides synthesized in a micrococcal nuclease-treated rabbit reticulocyte lysate programmed with various mRNA fractions and separated on a sodium dodecyl sulfate-polyacrylamide slab gel. Translation was performed in a reaction mixture (30 μ l) containing 10 μ l of reticulocyte lysate, 5 μ l of [³⁵S]methionine (700 to 900 Ci/mmol; New England Nuclear Corp.), 1 to 5 μ l of hybridization-selected RNA, 1 mM magnesium acetate, 83 mM potassium acetate, 30 μ g of rat liver tRNA per ml, 0.5 mM spermidine, 25 mM creatine phosphate, 40 μ g of creatine phosphokinase per ml, 1 mM ATP, 0.2 mM GTP, 1 mM dithiothreitol, and 50 μ M each of 19 unlabeled amino acids. Reaction mixtures were incubated at 30°C for 1 h and analyzed by electrophoresis on 25-cm-long, 10 to 18% sodium dodecyl sulfate-polyacrylamide gradient gels at 14 mA for 20 h. Gels were subjected to fluorography (4). ¹⁴C-labeled proteins used as molecular weight markers (New England Nuclear Corp.) were phosphorylase b (93K), bovine serum albumin (69K), ovalbumin (46K), carbonic anhydrase (30K), β -lactoglobulin (18.3K), and cytochrome c (12.3K). Lanes: (A) Total late mRNA; (B) no exogenous mRNA; (C) uninfected KB cell polyadenylic acid-containing RNA; (D) total early Ad2 mRNA; (E) Ad2 E4-specific mRNA. (B) Comparison of [³⁵S]methionine-labeled polypeptides synthesized in a micrococcal nuclease-treated rabbit reticulocyte lysate and a wheat germ lysate programmed with E4 mRNA. The wheat germ lysate kit was purchased from Bethesda Research Laboratories, and polypeptides were labeled with [³⁵S]methionine. Lanes: (A) Background polypeptide synthesis in a reticulocyte lysate; (B) E4 mRNA in a reticulocyte lysate; (C) E4 mRNA in a wheat germ lysate; (D) background polypeptide synthesis in a wheat germ lysate. (C) Hybrid arrested translation of E4 mRNA. Lanes A to E, in vitro translation in a reticulocyte lysate: (A) no exogenous mRNA; (B) E4 mRNA (0.4 μ g) hybridized to cloned Ad2 *Hind*III-F DNA (1.0 μ g); (C) E4 mRNA (0.4 μ g) hybridized to cloned Ad2 *Hind*III-F DNA (2.0 μ g); (D) E4 mRNA (0.4 μ g) hybridized to cloned *Hind*III-F DNA, then heated at 100°C for 1 min; (E) untreated E4 mRNA. Lanes F to J, in vitro translation in a wheat germ lysate: (F) no exogenous mRNA; (G) same as (B); (H) same as (C); (I) same as (D); (J) same as (E).

plete Ad2 genome. Polypeptides with apparent molecular weights of 70K to 72K, 55K, 40K to 50K, 35K, 23K, 22K, 21K, 18K, and 15K were resolved. The molecular weights of these polypeptides were estimated on the basis of their mobility relative to ^{14}C -labeled polypeptide standards (not shown). These polypeptides appear to be specific to Ad2 since neither reticulocyte endogenous mRNA (lane B) nor uninfected KB mRNA (lane C) directed their synthesis. Lane E shows the polypeptides synthesized with mRNA complementary to *EcoRI*-C (mp 89.7 to 100). Five major polypeptides of 35K, 23K, 22K, 21K, and 18K were visualized. Several minor polypeptides of 12K to 17K were also observed (lane E), but these were not reproducibly detected in other experiments, and therefore we do not know their significance. As expected, these polypeptides comigrated with many of the polypeptides that were translated from mRNA complementary to the complete genome (lane D).

The E4-specific mRNA was also translated in a wheat germ lysate to identify polypeptides that may comigrate with the globin synthesized in the reticulocyte lysate, and to attempt to identify additional E4 polypeptides. Figure 1B compares the polypeptides synthesized in the two lysates, using E4 mRNA selected by hybridization with cloned *HindIII*-F (mp 89.5 to 97.3). As shown in lane C, a prominent 11K polypeptide was synthesized in the wheat germ lysate; this polypeptide probably was also synthesized in the reticulocyte extract, but was obscured by the globin band (lane B). Most of the E4 polypeptides (23K, 22K, 21K, and 18K) were synthesized in both the reticulocyte and wheat germ lysates (lanes B, C). However, the 35K polypeptide appeared not to be synthesized or was synthesized in very low amounts in wheat germ lysate. Another minor polypeptide, 22.5K, was synthesized in wheat germ lysate but not in the reticulocyte lysate (lane C). We do not know why the two lysates differ in their ability to synthesize the 35K and 22.5K polypeptides. In this experiment, several minor polypeptides ranging from 24K to 30K were observed; these were not detected in other experiments and therefore their significance is unclear. Our results indicate that at least six polypeptides (35K, 23K, 22K, 21K, 18K, and 11K) are coded by E4.

To confirm the above conclusion that at least six polypeptides are coded by E4, DNA-mRNA hybrid arrested cell-free translation was performed (21). The principle of this procedure is that mRNA in hybrid form with its cDNA is not translated in cell-free systems, and that heat dissociation of the hybrid restores that translational activity (21). As shown in Fig. 1C, cloned *HindIII*-F (mp 89.5 to 97.3) fragment (1.0 to 2.0

μg) blocked or greatly reduced the translational activity of E4-specific mRNA (0.4 to 0.6 μg) in both the reticulocyte (lanes B, C) and wheat germ (lanes G, H) lysates. All of the E4 polypeptides (35K to 11K) were observed again after the hybrids were heat dissociated at 100°C for 1 min and then translated; that is, the same polypeptides were translated from the heat-dissociated hybrids (lanes D, I) as from mRNA not subjected to this process. (lanes E, J). Other restriction fragments such as *EcoRI*-A (E1 plus E5), *EcoRI*-B (E2), or *EcoRI*-D (E3) did not affect the synthesis of these polypeptides (not shown). These results confirm that all of these polypeptides are specified by E4.

The E4 polypeptides translated in vitro were further analyzed by 2D gel electrophoresis in an attempt to resolve possible polypeptides that comigrate on one-dimensional gels and to compare the positions of the in vitro E4 polypeptides with those of the in vivo E4 polypeptides resolved by 2D gel electrophoresis (6). Figure 2 shows the polypeptides translated from E4-specific mRNA (indicated by arrows). The most prominent spot was an 11K polypeptide, which migrated to the same position as did an 11K polypeptide synthesized in vivo (6). Therefore, the 11K polypeptide synthesized in vitro corresponds to the E4-11K polypeptide from Ad2-infected KB cells characterized by immunoprecipitation and 2D gel electrophoresis (6).

Five polypeptides in the 23K to 18K range were observed (arrows) that presumably correspond to the 23K to 18K polypeptides observed on the one-dimensional gel. Ad2-specific early polypeptides located at approximately the same position on 2D gels are also resolved from extracts labeled in vivo (6). Therefore, these in vitro polypeptides are synthesized in vivo. Two additional minor polypeptides were detected in the corresponding range on 2D gels of in vivo-labeled extracts. The seven in vivo polypeptides form a family of related polypeptides (6), as do the 23K to 18K in vitro polypeptides (see below). The 35K polypeptide, which is another member of this family, is not shown in Fig. 2 because translation was done in the wheat germ lysate.

Two additional polypeptides, 10K and 9K, were detected on the 2D gel (Fig. 3). These may represent additional polypeptides coded by E4.

To examine the sequence relationships among the E4 polypeptides, the major polypeptide bands were cut out from the gels in Fig. 1 and 2 and peptide mapped using trypsin (9, 11) (Fig. 3). Based on the tryptic peptide patterns, a family of related polypeptides appears to exist which includes 35K (Fig. 3A), 23K (B), 22K (C), 21K (D), and 18K (E); these polypeptides share the prominent [^{35}S]methionine tryptic peptides

FIG. 2. Fluorograph of 2D gel electropherogram of [^{35}S]methionine-labeled E4 polypeptides translated in a wheat germ lysate. Samples were prepared for isoelectric focusing, and the first-dimensional gels were run according to the method of O'Farrell (19) with minor modifications. The pH gradient of the gels was measured with a Bio-Rad electrode after the focusing was 4.5 to 6.1. The second-dimensional gels were run simultaneously on 10 to 18% sodium dodecyl sulfate-polyacrylamide gels using a DALT apparatus (Electro-Nucleonics, Oak Ridge, Tenn.) as described by Anderson and Anderson (1). After electrophoresis, the gels were subjected to fluorography. ^{14}C -labeled molecular weight markers included in the samples were (a) phosphorylase b (93K), (b) bovine serum albumin (68K), (c) ovalbumin (46K), and (d) β -lactoglobulin (18.3K).

numbered 1, 2, and 3 and possibly some other minor peptides. The major 11K (Fig. 3F), which was translated in the wheat germ lysate, has quite a different peptide map. It lacks the peptides 2 and 3 found in the 18K to 35K family. It is possible, however, that peptide b of 11K may correspond to peptide 1 of the 35K to 18K family, because when mixtures of tryptic digests of the 11K and 22K polypeptides were analyzed, spot 1 of the 22K seemed to comigrate with spot b of 11K (Fig. 3G). Thus, a portion of 11K may share sequences with the 35K to 18K family, although the comigration of peptides 1 and b may be merely coincidental.

We have shown above that polypeptides of 35K, 23K, 22K, 21K, 18K, and 11K are translated in a rabbit reticulocyte lysate or a wheat germ lysate, or both, with E4-specific mRNA. Hybrid arrested translation experiments confirmed that these polypeptides are coded by E4. Polypep-

tides of 10K and 9K were also detected on 2D gels, and these may also be coded by E4. Some of the E4 polypeptides that we have detected are probably the same as those described by Lewis and co-workers (13, 18). However, the 35K polypeptide, which is one of the major E4 polypeptides, has not been previously reported.

Peptide maps indicate that the 35K, 23K, 22K, 21K, and 18K polypeptides form a family of related polypeptides. Peptide maps of the 11K polypeptide are different from those of the 35K to 18K family. However, one tryptic methionine peptide of the 11K and the 35K to 18K polypeptides comigrated, raising the possibility that the 11K may be partially related to the 35K to 18K family.

We have previously shown that seven Ad2 early polypeptides with apparent molecular weights ranging from 26K to 21K and 11K are synthesized *in vivo* and can be resolved by 2D gel electrophoresis (6). We further showed that these polypeptides have peptide maps similar to those of one another and to a 22K polypeptide translated from E4-specific mRNA (6). Five members of the *in vitro* 35K to 18K family that we have described in this report migrate to approximately the same position on 2D gels as do those *in vivo* polypeptides, and they have similar peptide maps. Therefore, the 23K to 18K and presumably the 35K polypeptides are bona fide polypeptides that are synthesized *in vivo*. Most likely these polypeptides are independent translation products, rather than products that are generated by proteolysis, because (i) they appear both *in vitro* and *in vivo* and (ii) they are synthesized *in vitro* during a 5-min pulse with [^{35}S]methionine and they do not change in intensity after a 60-min chase with excess unlabeled methionine (Matsuo et al., unpublished data). The *in vitro* 11K polypeptide migrates to the same position on 2D gels as does an *in vivo* 11K polypeptide (6), and it has a similar peptide map; thus, the *in vitro* 11K is also a bona fide polypeptide that is synthesized *in vivo*. The 11K polypeptide is immunoprecipitated by antisera against 8617 cells (6, 22), an Ad2-transformed rat cell line that retains E4 but not E2 or E3, which is consistent with the conclusion that the 11K is coded by E4.

The finding that the E4 polypeptides fall into two groups, 35K to 18K and 11K (Fig. 3), is interesting in the context of the organization of the genes in E4. E4 encodes at least six (8, 17) and as many as nine (11a) partially overlapping mRNA's with different splicing patterns. Conceivably, each mRNA may encode one of the 35K to 18K polypeptides or the 11K polypeptide. The overlapping (common) portions of the mRNA's could code for the common portions of the polypeptides, and the nonoverlapping por-

FIG. 3. Tryptic peptide maps of E4 polypeptides purified from the gel shown in Fig. 1A. Bands on dried gels were cut out, washed with 10% methanol, dried, and digested with 200 μ l of trypsin (50 μ g/ml, Worthington) in 50 mM NH_4HCO_3 at 37°C for 2 h, followed by incubation with 500 μ l of water overnight. Supernatants were lyophilized three times and dissolved in 10 μ l of electrophoresis buffer (acetic acid-formic acid-water, 15:5:80) and resolved on a 10-by-10-cm cellulose-coated thin-layer plates by electrophoresis at pH 1.7 in the first dimension and ascending chromatography in butanol-pyridine-acetic acid-water (32.5:25:5:20) in the second dimension. Plates were coated with molten 0.4% 2,5-diphenyloxazole (PPO) in methylnaphthalene (5) and autoradiographed at -70°C for 2 to 6 weeks. (A) E4, 35K; (B) E4, 23K; (C) E4, 22K; (D) E4, 21K; (E) E4, 18K; (F) E4, 11K synthesized in a wheat germ lysate; (G) mixture of 22K and 11K.

tions of the mRNA's could encode regions that are unique to each polypeptide. Thus, a limited set of DNA sequences could be used to generate, via splicing, a variety of polypeptides that differ in overall structure and therefore in function. A similar situation may also exist in Ela, where overlapping mRNA's of 13S, 12S, and 9S

(3, 8, 11a, 17) encode several related polypeptides (10-12). Other examples would be the early and late regions of papovaviruses. Clearly, this type of arrangement would greatly expand the information content of a given stretch of DNA, which should be of survival value to the virus that has genome packaging constraints. It re-

mains to be established, however, whether the different E4 polypeptides are in fact encoded by different mRNA's, how the initiation and termination codons and reading frames are arranged in the E4 mRNA's so as to generate related but different polypeptides, and whether the E4 polypeptides actually have different functions.

We thank H. Thornton for assistance with cell culture and virus preparation, M. Pursley for excellent technical assistance, and K. Brackmann for providing advice.

This work was supported by Public Health Service grants CA-29561 and CA-24710 and Cancer Center CORE grant CA-25843; W.S.M.W. was supported by Public Health Service Research Career Development Award K04-CA-00650; M.G. was supported by Public Health Service Research Career Award 5K06-AI-04739; all grants were from the National Institutes of Health.

LITERATURE CITED

- Anderson, N. L., and N. G. Anderson. 1978. Analytical techniques for cell fractions. XXII. Two-dimensional analysis of serum and tissue proteins: multiple gradient-slab gel electrophoresis. *Anal. Biochem.* 85:341-354.
- Baker, C. C., and E. Ziff. 1979. Biogenesis, structures, and sites of encoding of the 5'-termini of adenovirus-2 mRNAs. *Cold Spring Harbor Symp. Quant. Biol.* 44:415-428.
- Berk, A. J., and P. A. Sharp. 1978. Structure of the adenovirus 2 early mRNAs. *Cell* 14:695-711.
- Bonner, W. M., and R. S. Laskey. 1974. A film detection method for ^3H -labeled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* 46:83-88.
- Bonner, W. M., and J. D. Stedman. 1978. Efficient fluorography of ^3H and ^{14}C thin layers. *Anal. Biochem.* 89:247-256.
- Brackmann, K. H., M. Green, W. S. M. Wold, M. A. Cartas, T. Matsuo, and S. Hashimoto. 1980. Identification and peptide mapping of human adenovirus type 2 induced early polypeptides isolated by two-dimensional gel electrophoresis and immunoprecipitation. *J. Biol. Chem.* 255:6772-6779.
- Büttner, W., Z. Veres-Molnar, and M. Green. 1976. Preparative isolation and mapping of adenovirus 2 early messenger RNA species. *J. Mol. Biol.* 107:93-114.
- Chow, L. T., R. T. Broker, and J. B. Lewis. 1979. Complex splicing patterns of RNAs from the early regions of adenovirus-2. *J. Mol. Biol.* 134:265-303.
- Elder, J. H., R. A. Pickett, J. Hampton, and R. A. Lerner. 1977. Radioiodination of proteins in single polyacrylamide gel slices. *J. Biol. Chem.* 252:6510-6515.
- Esche, H., M. B. Mathews, and J. B. Lewis. 1980. Proteins and messenger RNAs of the transforming region of wild-type and mutant adenovirus. *J. Mol. Biol.* 142:399-417.
- 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* 97:278-286.
- Green, M., W. S. M. Wold, and W. Büttner. 1981. Integration and transcription of group C human adenovirus sequences in the DNA of five lines of transformed rat cells. *J. Mol. Biol.* 151:337-366.
- Halbert, D. N., D. J. Spector, and H. J. Raskas. 1979. In vitro translation products specified by the transforming region of adenovirus type 2. *J. Virol.* 31:621-629.
- Harter, M. L., and J. B. Lewis. 1978. Adenovirus type 2 early proteins synthesized in vitro and in vivo: identification in infected cells of the 38,000- to 50,000-molecular-weight protein encoded by the left end of the adenovirus type 2 genome. *J. Virol.* 26:736-749.
- Hashimoto, S., and M. Green. 1979. Methylated 5'-terminal caps of adenovirus type 2 early mRNA: evidence for at least six 5'-termini. *Virology* 94:254-272.
- Hashimoto, S., and M. Green. 1980. Adenovirus 2 early messenger RNA—genome mapping of 5'-terminal RNase T1 oligonucleotides and heterogeneity of 5'-termini. *J. Biol. Chem.* 255:6780-6788.
- Hashimoto, S., M. H. Pursley, and M. Green. 1981. Nucleotide sequences and mapping of novel heterogeneous 5'-termini of adenovirus 2 early region 4 mRNA. *Nucleic Acids Res.* 9:1675-1689.
- Kitchingman, G. R., and H. Westphal. 1980. The structure of adenovirus 2 early nuclear and cytoplasmic RNAs. *J. Mol. Biol.* 137:23-48.
- Lewis, J. B., J. F. Atkins, P. R. Baum, R. Solem, T. F. Gesteland, and C. W. Anderson. 1976. Location and identification of genes for adenovirus type 2 early polypeptides. *Cell* 7:141-151.
- O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* 250:4007-4021.
- Parsons, J. T., and M. Green. 1971. Biochemical studies on adenovirus multiplication. XVII. Resolution of early virus specific species in Ad2 infected and transformed cells. *Virology* 45:154-162.
- Paterson, B. M., B. E. Roberts, and E. L. Kuff. 1977. Structural gene identification and mapping by DNA-mRNA hybrid-arrested cell-free translation. *Proc. Natl. Acad. Sci. U.S.A.* 74:4370-4374.
- Pelham, H. R. B., and R. J. Jackson. 1976. An efficient mRNA-dependent translation system from reticulocyte lysates. *Eur. J. Biochem.* 67:247-256.
- Wold, W. S. M., and M. Green. 1979. Adenovirus type 2 early polypeptides immunoprecipitated by antisera to five lines of adenovirus-transformed cells. *J. Virol.* 30:297-310.