Characteristics of a Human Cell Line Transformed by DNA from Human Adenovirus Type 5

By F. L. GRAHAM* AND J. SMILEY†
Departments of Biology and Pathology, McMaster University, Hamilton, Ontario, Canada

W. C. RUSSELL AND R. NAIRN‡
Divisions of Virology and Biochemistry, National Institute for Medical Research, Mill Hill, London, NW7 1AA, U.K.

(Accepted 10 February 1977)

SUMMARY

Human embryonic kidney cells have been transformed by exposing cells to sheared fragments of adenovirus type 5 DNA. The transformed cells (designated 293 cells) exhibited many of the characteristics of transformation including the elaboration of a virus-specific tumour antigen. Analysis of the polypeptides synthesized in the 293 cells by labelling with 35S-methionine and SDS PAGE showed a variable pattern of synthesis, different in a number of respects from that seen in other human cells. On labelling the surface of cells by lactoperoxidase catalysed radio-iodination, the absence of a labelled polypeptide analogous to the 250 K (LETS) glycoprotein was noted. Hybridization of labelled cellular RNA with restriction fragments of adenovirus type 5 DNA indicated transcription of a portion of the adenovirus genome at the conventional left hand end.

INTRODUCTION

Transformation of cultured cells by animal viruses has been demonstrated using a variety of DNA viruses and cells. However, most transformation studies have utilized cells of non-human origin, with rodent cells the most commonly used. This is especially true in the case of human adenoviruses; with the exception of one early report (Todaro & Aaronson, 1968) transformation of human cells by adenoviruses has not been observed, and isolation of adenovirus transformed human cell lines has not been reported. The difficulty in obtaining transformation of human cells with human adenoviruses is presumably due, in large part, to the fact that exposure of permissive cells to adenoviruses normally leads to a lytic infection with the production of large numbers of infectious virions. However, a technique (the ‘calcium technique’) has been developed for infecting cells with virus DNA (Graham & van der Eb, 1973a; Graham, Veldhuisen & Wilkie, 1973) which appears to be particularly

* Research Scholar and † Research Student of the National Cancer Institute of Canada.
‡ Present address: Department of Microbiology and Immunology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York, N.Y. 10461, USA.
suitable for transforming cells with DNA (Graham & van der Eb, 1973b; Graham, van der Eb & Heijneker, 1974). Of special importance in the context of transformation of permissive cells was the observation that transformation of cells could be induced with fragmented virus DNA (Graham & van der Eb, 1973b; Graham et al. 1974). Using this approach, transformation of human cells has been induced by sheared Ad 5 DNA and an Ad 5 transformed human cell line (293) has been established. This report describes some of the properties of these 293 cells.

**METHODS**

**Virus and cells.** Adenovirus type 5 (strain Ad 75) was grown and purified as described previously (Graham & van der Eb, 1973a) and extraction of DNA from purified virions was carried out according to van der Eb, van Kesteren & van Bruggen (1969). Ad 5 DNA was sheared by forcing DNA solutions (100 to 200 µg DNA/ml in 0.1 × SSC) repeatedly through a 22 gauge needle (inside diam. 0.72 mm). The resulting DNA had a modal sedimentation coefficient of 22S as determined by band sedimentation in an analytical ultracentrifuge, indicating a modal mol. wt. of 8 × 10^6 or about 1/3 the size of intact Ad 5 DNA.

Primary or early passage secondary human embryonic kidney (HEK) cells prepared by standard techniques were grown in Eagle’s minimum essential medium (MEM) supplemented with non-essential amino acids and 10% calf serum and were used in transformation studies at subconfluency. Exposure of cells to virus DNA was carried out using the calcium technique essentially as described by Graham & van der Eb (1973a, b). About 3 days after exposure to DNA the cultures were switched to low calcium ion medium (Freeman et al. 1967) and examined periodically for the presence of colonies of transformed cells. After establishment of the cell line the cells were grown routinely in Eagle’s medium containing 10% calf serum supplemented with tryptose phosphate broth (ETC) (see text).

**Fluorescent antibody techniques.** Fluorescent antibody techniques using the indirect method have been described previously (Hayashi & Russell, 1968).

**Antisera.** A rabbit P antiserum was prepared as previously described (Russell et al. 1967). Hamster tumour sera were obtained from hamsters bearing tumours induced by transplanting hamster cells transformed by sheared virus DNA (Graham et al. 1974) into baby hamsters. Sera were also obtained from hamsters bearing tumours induced by similarly transplanting hamster cells (H 14a) transformed by an adenovirus temperature sensitive mutant (Williams, 1973). In this latter case it is known that these hamster cells only contain about 35% of the left hand end of the virus genome (Sambrook et al. 1974).

**Cell labelling and polyacrylamide gel electrophoresis (PAGE).** Monolayers of cells in 1 oz glass bottles were labelled by removing medium and replacing with 0.5 ml of medium lacking methionine but containing ^35S-methionine (20 µCi/ml, 200 to 350 Ci/mmole, Radiochemical Centre, Amersham, Bucks) and incubating at 38.5 °C for 1 h or as appropriate. Cells were then scraped off and washed in phosphate buffered saline prior to suspension in a small volume of hypotonic buffer (5 mm-tris/HCl, pH 7.8). Samples were denatured by boiling in sodium dodecyl sulphate (SDS) and analysed by PAGE using the discontinuous buffer system containing SDS and stained with Coomassie or submitted to autoradiography as described previously (Russell & Blair, 1977).

**Iodination procedure.** Cells were grown in Glasgow-modified Eagle’s medium containing 10% foetal calf serum supplemented with a non-essential amino acid mixture (Gibco Biocult) as monolayers in 60 mm plastic Petri dishes and iodinated using the lactoperoxidase catalysed method as described elsewhere (Meager, Nairn & Hughes, 1975).
Adenovirus transformed human cells

Cytochemistry. Cells were grown as monolayers on coverslips in plastic Petri dishes, fixed in ethanol and stained with Giemsa in the standard manner or with phenanthrenequinone as indicated elsewhere (Russell, Brodaty & Armstrong, 1971).

Restriction enzyme digestion and electrophoresis. Endonuclease endo R.Hin d III was purchased from Bethesda Research Laboratories. Ad 5 DNA was digested with excess endonuclease at 37 °C for 3 h in 20 mM-tris-HCl, pH 7.4, 60 mM-NaCl, and 7 mM-MgCl2. Samples were then adjusted to contain 0.5 % SDS, 0.005 % bromophenol blue, 5 % glycerol and 20 µl samples containing 2 µg virus DNA were loaded on to 16 mm cylindrical 1 % agarose gels. The gels were electrophoresed at 2 V/cm for 12 h at room temperature in the electrophoresis buffer of Sharp, Sugden & Sambrook (1973), lacking ethidium bromide. Gels were stained with ethidium bromide and the DNA bands were visualized and photographed under shortwave ultraviolet illumination.

RNA-DNA hybridization. Monolayers were labelled for 1 h with 50 µCi/ml 3H-uridine (New England Nuclear, 20 Ci/mmol) and whole cell RNA was extracted by the hot phenol SDS-method (Warner et al. 1966). After ethanol precipitation the RNA was dialysed against 0.1 × SSC, then hybridized to unlabelled Ad 5 DNA restriction fragments which had been previously transferred from agarose gels to nitrocellulose strips by the method of Southern (1975). Hybridization was done at 65 °C in 2 × SSC plus 0.1 % SDS and 100 µg/ml yeast RNA for 20 h. Washing, ribonuclease digestion and fluorography were done as described by Southern (1975) except that the X-ray film was flash hypersensitized (Laskey & Mills, 1975) before use. The resulting negatives were scanned with a Joyce–Loebl microdensitometer.

RESULTS

Transformation of HEK cells

Using procedures similar to those used successfully to transform rat and hamster kidney cells (Graham et al. 1974) a total of 8 transformation experiments have been carried out with, on average, 20 cultures of HEK cells per experiment. For reasons which are not completely clear, transformation of human cells by Ad 5 DNA was extraordinarily inefficient compared to results with rat or hamster cells even though the DNA had been sheared to eliminate infectivity. Only two experiments with HEK cells were successful and in each of these a single morphologically transformed colony was observed approximately 1 month after exposure of the cultures to Ad 5 DNA. Attempts to isolate these colonies failed but in one case the original dish was retained and eventually (about day 75) a few transformed cells could again be observed in the area of the dish where the colony first developed. Since the culture still contained a rather dense monolayer of normal cells the serum content was reduced to 2 % in an attempt to select the transformed cell phenotype. At day 99 the culture was transferred to a large plastic bottle in medium containing 2 % calf serum (CS) and by this time a significant proportion of the cells now assumed the transformed cell phenotype. The culture was again split (1 to 2) at days 120 and 142 and at day 151 enough cells were obtained to freeze 3 ampoules in liquid nitrogen (passage 4). At each transfer and subsequent incubation in 2 % CS the proportion of transformed cells (based on their characteristic morphology) increased. The normal cells were not completely eliminated until about passage 6, after which the serum concentration was increased to 10 % CS. At passage 13, the cells entered a ‘crisis’ phase which lasted until passage 16 (from day 300 to about day 400) and which was characterized by little or no growth. The growth rate increased rather sharply after passage 16 and the cells could thereafter be split 1 in 3 about twice weekly (Fig. 1).
Fig. 1. Growth curve of 293 cells. The points on the curve represent the area of the 293 cell monolayer at each consecutive passage (taking into account removal of cells for freezing, experiments, etc.) except that the first point was calculated assuming an area of $10^{-2}$ cm$^2$ for a single cell.

This growth pattern appears to be reproducible for these cells in the sense that, in two additional instances, sublines started from ampoules of cells frozen at passage 6 entered a crisis around passage 13 or 14. In one case the subline failed to recover, in the other, growth resumed 60 to 80 days later. The cells have since been maintained in Eagle’s medium (or medium containing reduced calcium ion concentration) supplemented with 10% calf serum and tryptose phosphate broth (ETC). Horse serum can be substituted for calf serum and appears to be equally suitable for cell growth. The cells have been subcultured more than 100 times and can presumably be considered an established line (293 cells).
Fig. 2. Staining properties of 293 cells. (a) Giemsa staining: ×220. (b) Phenanthrenequinone staining: ×1100. (c) Fluorescent antibody staining with tumour serum: ×1000. (d) Fluorescent antibody staining with P antiserum: ×3500.
Morphology of 293 cells

The cells exhibited a typical adeno-transformed cell phenotype with a tendency to continue dividing after reaching confluency and to grow in islands or clumps. Like adeno-transformed rat and hamster cells, 293 cells were grossly epithelioid in character (as was the other transformed colony observed in 8 attempts to transform HEK cells) but were, on average, noticeably larger than most other adeno-transformed cells, showing a wide variation in cell size (Fig. 2a). In some cases, the cell nuclei appeared swollen and frequent heterokaryons were noted. Another similarity was the ability of 293 cells to grow equally well in medium containing or lacking calcium ions or to grow in medium containing reduced serum concentrations. A characteristic feature of many of the cells was a dense ovoid body which could readily be detected by staining with phenanthrenequinone and which generally seemed to be located at or near the nuclear membrane (Fig. 2b). The human character of the cells was confirmed by the chromosome banding pattern with quinacrine mustard and were quasi tetraploid when tested at passage 8 (P. Pearson, personal communication). Chromosome spreads were again prepared for cells at passage 38 as well as for a subline of 293 cells (293 N) established from a tumour induced in a nude mouse. In both cases the resulting karyotype was that of human cells though the average number of chromosomes in 293 N cells appeared to be significantly lower than for the parental line. (Similar observations have been made by P. Gallimore, personal communication.)

Superinfection with adenoviruses

One characteristic of 293 cells which makes them of considerable usefulness for studies with adenoviruses is that they can be superinfected by Ad 5 as well as other human adenovirus serotypes. Indeed, 293 cell monolayers were excellent substrates for plaque titrations of adenovirus serotypes 1, 2, 5 and 7 as well as type 12 which we have been unable to plaque on other established human cell lines. Plaque assays with Ad 5 resulted in plaques appearing several days earlier on 293 monolayers than on HeLa or KB monolayers though the final numbers of plaques were similar.

Another interesting property of 293 cells is that Ad 5 DNA titrated on these cells (using the calcium technique) is 10 to 50 times more infectious than when titrated on KB cells or primary HEK cells (F. L. Graham, unpublished observations). The reasons for this are unknown, but could possibly be related to the fact that, as discussed in following sections, 293 cells express one or more Ad 5 specific products.

Oncogenicity

The ability of 293 cells to induce tumours was tested by injection of 1 to 2 × 10⁷ cells subcutaneously into 5 to 6-week-old ‘nude’ mice. After 6 to 8 weeks no tumours were visible and the animals were re-injected. After approx. 15 to 20 weeks from the time of the first injection, tumours became apparent in a small proportion (3/20) of treated animals. (An outbreak of murine hepatitis prevented the course of the experiment from being followed longer than 25 to 30 weeks). In contrast, Ad 5 DNA transformed rat or hamster cells induced tumours in 90 to 100% of injected mice within 3 to 6 weeks. One animal exhibiting a 293 cell-induced tumour was sacrificed at 16 weeks and the tumour was removed, minced and put into culture. The resulting cells (line 293 N) were somewhat similar to the original 293 cells but grew slightly faster and reached greater cell densities. The 293 N cells like the parental cells, were capable of growth in medium with low concentrations of calcium ions.
Adenovirus transformed human cells

In addition the 293 N cells continued to express Ad 5 specific T antigen(s) as detected by indirect immunofluorescence.

Fluorescent antibody experiments

Monolayers of 293 cells on coverslips were reacted with hamster tumour antisera and with rabbit antiserum against the adenovirus type 5 P antigen. The latter antiserum reacts with early antigens but mainly against the major early 72 K single-stranded DNA binding protein (Russell et al. 1967; Russell & Skehel, 1972; Russell & Blair, 1977). With the tumour antiserum fluorescent nuclear dots were noted in about 20% of the cells; however, the most characteristic finding was a large fluorescent fleck apparently in association with the nuclear membrane (Fig. 2c). The same feature was also noted on reacting the cells with the rabbit P antiserum. In this case (Fig. 2d) the staining was less intense and only about 5% of the cells showed nuclear dots. On staining HeLa cells infected with adenovirus type 5 in the presence of cytosine arabinoside with these sera, the tumour antiserum exhibited minimum nuclear fluorescence and occasional cytoplasmic flecks in association with the nuclear membrane. On the other hand the P antiserum showed intense characteristic staining as fibres and dots on all the cells as previously described (Hayashi & Russell, 1968).

The fluorescent staining of the 293 cells could be significantly reduced by adsorbing both the tumour and P antisera with extracts of the 293 cells but not with extracts of control cells. No fluorescent antigens could be detected in the 293 cells when the cells were reacted with adenovirus type 5 hexon or fibre antisera.

Polypeptide synthesis in 293 cells

Monolayers of 293 cells were pulse labelled with 35S-methionine for 1 h and for longer periods. In some experiments the 1 h pulses were 'chased' for about 18 h. The extracts were submitted to analysis by SDS polyacrylamide gel electrophoresis followed by autoradiography. The labelling patterns were compared to those obtained from other human cell lines labelled in parallel in the same way. In the course of about twenty experiments carried out at a number of different passage levels the 293 cells were compared with primary and secondary human embryonic kidney cells, with other epithelioid cells such as HeLa and KB cells and with human fibroblast lines such as WI-38 and MRC-5. The labelling patterns seen with the control cell lines remained substantially constant whereas the labelling patterns seen with the 293 cells showed considerable variation from experiment to experiment. This variation did not seem to be a reflection of the growth state of the cell (i.e. whether in log or lag phase) or of the passage number. The differences from the other human cell lines were evident both as a diminution in labelling of some polypeptides and the appearance of 'new' bands in the autoradiograms. It was also noted that the labelling patterns changed significantly after 'chasing' the 293 cells, suggesting that some processing of the polypeptides was occurring. Fig. 3 shows a typical autoradiogram obtained from labelled extracts of 293 cells and infected and uninfected HeLa cells. When compared to the uninfected cell pattern it will be seen that there is some diminution in the labelling of high mol. wt. polypeptides particularly at mobilities corresponding to the 250 K/LETS (large, external transformation sensitive) glycoprotein (Hynes, 1973). It is also clear that there is no detectable synthesis of the 72 K polypeptide in the 293 cells. The latter polypeptide is the major polypeptide synthesized early in infected cells and is heavily phosphorylated (Russell & Skehel, 1972; Russell & Blair, 1977). The major difference in the labelling patterns is the apparent synthesis in the 293 cells of a polypeptide of mol. wt. about 68 K which can also be seen early in adenovirus-infected cells. As yet, however, there is no clear indication whether this is a virus-
Fig. 3. Autoradiograms after SDS PAGE of extracts of HeLa and 293 cells after labelling with $^{35}$S-methionine for 1 h at the post infection (V) times shown. Uninfected HeLa cells (C) and 293 cells were similarly labelled for 1 h. Iodinated type 5 virus ($^{125}$I V) and extracts of $^{32}$P-labelled infected cells ($^{32}$P 16 V – see Russell & Blair, 1977) were run in parallel as markers. On the right of the figure the labelled bands corresponding to the major structural polypeptides are indicated. On the left the mobilities corresponding to LETS and 72 K polypeptides are marked. The remaining arrows indicate the differences in mobilities between the 293 cells and the uninfected HeLa cells (C). Origin of electrophoresis is at the top of the figure.

coded or an induced cellular polypeptide (cf. Saborio & Oberg, 1976). The other notable difference between the 293 cells and uninfected HeLa cells is the apparent synthesis of a number of smaller mol. wt. polypeptides. Attempts to detect differences in phosphorylated polypeptides between the 293 cells and other cells by labelling with $^{32}$P-orthophosphate (Russell & Blair, 1977) were unsuccessful.

Cell surface labelling patterns

On labelling surface components of the 293 cells by lactoperoxidase catalysed $^{125}$I iodination a number of differences could be seen when compared to the pattern obtained with normal human embryo fibroblasts (HEF; Fig. 4). Most of the high mol. wt. iodinated polypeptides of the HEF cells are absent from the 293 cells and concomitantly other smaller
Adenovirus transformed human cells

Fig. 4. Cell surface labelling profile of 293 cells in comparison to human embryo fibroblast cells (HEF). Cells were iodinated, denatured in SDS, and equal protein samples run on adjacent tracks of a discontinuous SDS-polyacrylamide slab gel. Purified reovirus was electrophoresed in parallel as a marker. The gels were sliced into 2 mm sections for gamma counting in a Packard Auto-gamma spectrometer. On the x-axis, 0 represents the start of the 7.5 % resolving gel. Electrophoresis is from left to right. \( \lambda_1 \) and \( \mu_2 \) are the reovirus structural polypeptides of 155 K and 72 K mol. wt. respectively.

labelled polypeptides appear to be more prominent in the transformed cells. In particular the absence of a polypeptide probably analogous to the 250 K/LETS glycoprotein of other systems is noted and this is consistent with results obtained with certain other adenovirus transformed cell lines (Meager et al. 1975).

RNA-DNA hybridization

In order to determine which region(s) of the Ad 5 genome are transcribed in 293 cells, the total cellular RNA labelled with \(^3\)H-uridine was hybridized to unlabelled Ad 5 DNA fragments generated by endo R.\( \text{Hin} \) d \( \text{III} \), which had previously been transferred from agarose gel to nitrocellulose strips by the blotter method of Southern (1975). Fig. 5 shows
Fig. 5. Hybridization of labelled 293 cellular RNA to Ad 5 DNA fragments generated by endo R. *Hind III*. Ad 5 DNA (2 μg) was digested with endo R. *Hind III* and electrophoresed through a 1% agarose gel as described in Methods. The DNA bands were photographed under ultraviolet light in the presence of 0.5 μg/ml ethidium bromide. The fragments were transferred to nitrocellulose strips, and then hybridized to $5 \times 10^7$ ct/min of 293 cellular RNA labelled with $^3$H-uridine. Radioactivity was detected by fluorography, and the resulting negatives scanned with a Joyce-Loebl microdensitometer. (a) Photograph of the ethidium bromide fluorescence of Ad 5 *Hind III* fragments separated in a 1% agarose gel. (b) Fluorogram of the nitrocellulose strip produced from the gel displayed in (a) after hybridization to labelled 293 cellular RNA. (c) Microdensitometer tracing of the fluorogram in (b).
that 293 RNA hybridizes to the \textit{Hin} d \textit{III} G and E fragments, which together constitute the extreme left hand 17\% of the genome (C. Mulder, personal communication). In order to resolve fragments F and G well it was necessary to run fragment I (corresponding to the extreme right hand 4\% of the genome) out of the gel. Consequently, it remains possible that 293 cells contain RNA derived from \textit{Hin} d \textit{III} I. It is of interest that we can detect no RNA derived from the \textit{Hin} d \textit{III} A fragment, which should contain the sequences coding for the 72 K DNA binding protein (Lewis \textit{et al.} 1976), assuming that the gene for the protein is located at the same position on both the Ad 2 and Ad 5 genomes. Presumably the RNA species detected are analogous to the left hand early strand specific RNAs detected in Ad 2 and Ad 5 infected and transformed cells (Sharp, Gallimore & Flint, 1974; Flint, Berget & Sharp, 1976; Flint \textit{et al.} 1976).

\section*{DISCUSSION}

These studies have shown that Ad 5 DNA fragments can transform human cells with an efficiency which is at least 2 logs lower than that which is routinely obtained with rat or hamster cells. Further, a line of human cells transformed by Ad 5 could be established by careful maintenance of cell cultures following exposure of HEK cells to sheared virus DNA. The reasons for the difficulty in transforming human cells with Ad 5 DNA are not known. One difficulty which did arise in transformation studies with HEK cells which was not a problem with rat kidney cells was that normal HEK cells tended to persist in DNA-treated cultures for long periods following exposure and formed rather dense monolayers. This could have resulted in growth inhibition of any transformed cells which might have been induced. Other differences between human and rat cells are the slow growth rate and clear ‘crisis’ phase exhibited by the Ad 5 transformed human cells, and their extremely low oncogenicity in nude mice. The 293 cells are similar to Ad 5 transformed rat and hamster cells in their epithelioid morphology, their ability to grow in medium containing low concentrations of calcium ions, and their ability to elaborate virus-specific T antigen. The 293 cells were found to contain a rather unusual feature in the presence of large ovoid bodies in the region of the nuclear membrane clearly seen on staining by phenanthrenequinone. This cytochemical technique has been used to detect basic proteins and appears to react specifically with arginine residues (Russell \textit{et al.} 1971). Whether these inclusions bear any relationship to the T antigen containing entities detected by fluorescence is not clear. Staining by the DAPI technique (Russell, Newman & Williamson, 1975) which can readily detect DNA in the cytoplasm indicates that these bodies do not contain detectable amounts of DNA (unpublished observations). Their nature and their relationship (if any) to the maintenance of the transformed state will require further study.

The fluorescent antibody results indicate that the cells probably contain information from the conventional left hand end of the adenovirus genome since a positive reaction was obtained with tumour serum from animals bearing tumours induced by cells which are known to contain only that portion of the genome (Sambrook \textit{et al.} 1974). This was confirmed by the RNA-DNA hybridization studies which detected the presence in 293 cells of mRNA complementary to the extreme left end of the Ad 5 genome. The results with the P antiserum suggest that these cells do not elaborate significant amounts of the major early adenovirus antigen (Russell, 1974) associated with the 72 K single stranded DNA binding protein, consistent with our failure in three separate experiments to detect any virus-specific mRNA complementary to the region (around 60 to 70\%) of the adeno genome known to code for the DNA binding protein (Lewis \textit{et al.} 1976). These studies also suggest that the P
antiserum, while reacting principally with the 72 K polypeptide (Russell & Skehel, 1972), also reacts with the other early, so called, 'T' antigen(s).

The analyses of the polypeptide synthesis in the transformed cells were somewhat variable and the patterns obtained were quite unlike other transformed cell lines examined (Russell, 1974). Whether this inherent instability in the pattern of synthesis is related to lack of control of gene expression or perhaps to heterogeneity in the cell population can only be decided by more detailed investigations and by cloning of the cells. Some of the polypeptides synthesized are similar in mobility in the electropherograms to those seen in cells infected early in the lytic system but immunoprecipitation experiments using both the P and T antisera have so far yielded inconclusive results. In all these studies there has been no evidence of the production of either capsid antigens or capsid polypeptides. Thus there is no indication of whether the variable results in these analyses reflect variable expression of virus or cellular genes. Three experiments on the virus RNA transcription pattern all gave consistent results, however, suggesting that the differences in polypeptide synthesis are not due to differences at the level of transcription of virus genes.

The iodination experiments gave results similar to those described in other adenovirus transformed cell systems (Meager et al. 1975), the large 250 K polypeptide (LETS glycoprotein) not being accessible to the iodination reaction in the 293 cells. A similar finding has been reported by Chen, Gallimore & McDougall (1976) using an immunofluorescent technique and it has been shown by these authors that there is a correlation between tumourogenicity and the lack of the LETS glycoprotein on the cell surface. It is also notable that the synthesis of a large mol. wt. polypeptide of similar mol. wt. to LETS was substantially reduced compared to HeLa cells. Thus, in the case of the 293 cells the lack of radio-iodination of the large 250 K polypeptide may result from an inhibition of its synthesis rather than its non-appearance at the surface of the transformed cell.

Investigations elsewhere (R. Weinmann, personal communication) using DNA-DNA hybridization techniques have indicated that the 293 cells contain 4 to 5 copies of the left hand 12% of the Ad 5 genome and about 1 copy of the right 10%. The RNA-DNA hybridization studies described here are consistent with these findings in that virus RNA specific for the left end of the virus genome was detected. However, at present we are unable to say whether the virus sequences from the right 4% of the genome are transcribed. Studies with adenovirus type 2 transformed rat cells have indicated that some cell lines can contain DNA sequences (corresponding to 'late' regions) which are apparently untranscribed (Sambrook et al. 1974; Flint & Sharp, 1976).

The 293 cell line described here is, to our knowledge, the only adenovirus transformed human cell line established to date. Our preliminary characterization of some of the properties of these cells suggests that they will be quite useful in studies with adenoviruses. In particular, the 293 cells can be readily superinfected with human adenovirus and recently have been utilized to select host range mutants of adenovirus type 5. Recombination tests have indicated that these host range mutations map within the left hand region of the genome and probably very close to the left hand end (Harrison, Graham & Williams, 1976), findings which are consistent with the properties of the cells described in this communication.

This work was supported in part by the National Cancer Institute of Canada. One of us (FLG) is indebted to the European Molecular Biology Organization for a short term Fellowship which allowed some of the work to be carried out at Mill Hill. Another (RN) was in receipt of an MRC scholarship for training in Research Methods. We are also pleased to acknowledge the excellent technical assistance of Mr John Wills and Miss Gunvanti Patel.
and the cooperation of Mr M. R. Young and Miss E. Brodaty in supplying photomicrographs. Dr J. Williams assisted us at an early stage of the investigations by supplying 293 cells which he had successfully adapted to grow in ETC. We thank Dr J. J. Skehel for supplying a sample of purified reovirus.

REFERENCES


(Received 25 November 1976)