Expression of Early Viral Gene Products in Adenovirus Type 12-infected and -transformed cells

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SUMMARY

We have analysed early viral gene products expressed in adenovirus type 12 (Ad12)-infected cells as well as in two Ad12-transformed hamster cell lines, and Ad12-induced rat tumour cell lines by cell-free translation of virus-specific RNA which was selected by hybridization to cloned restriction endonuclease fragments of virus DNA. Proteins synthesized in vitro were analysed by one- and two-dimensional gel electrophoresis. It was found that RNA encoded by early region E1A directs the synthesis of at least eight polypeptides with apparent mol. wt. 38K, 36K, 30K, 28K, 26K, 25K, 24K and 22K. All these proteins are related to each other. E1B-specific RNA directs the synthesis of three proteins: 59K, 19K and 17K. Early region E2a codes for a 61K polypeptide which probably represents the single-strand DNA-binding protein of Ad12. RNA complementary to region E3 directs the synthesis of a 16K protein, and RNA transcribed from region E4 the synthesis of polypeptides with mol. wt. 20K, 18K and 11.5K. We have mapped a 67K polypeptide into the region within 11 to 28 map units (E2b). The analysis of proteins directed by virus-specific RNAs prepared from two Ad12-transformed hamster cell lines (T637, HA12/7) and one Ad12-induced rat tumour line (RBT12/3) showed that early regions E1 and E4 are expressed in all three Ad12-transformed cell lines. RNA transcribed from early regions E2 and E3 have been detected in lines T637 and RBT12/3. The virus RNA prepared from the Ad12-transformed cell lines directed synthesis of polypeptides with mol. wt. very similar to those of early virus proteins from infected cells. However, in all three Ad12-transformed cell lines mentioned above we have found RNAs which directed the synthesis of additional polypeptides of early regions E1 (34K) and E4 (25K, 24K) not detected in infected cells. The DNA sequence between 11 and 28 map units (coding for the 67K protein) is not expressed in the Ad12-transformed cells.

INTRODUCTION

Human adenoviruses, especially types 2, 5 and 12, have been the subject of extensive studies because of their ability to cause morphological transformation of cultured cells, or in the case of Ad12, to induce tumours in animals. Transformation of cells with purified restriction endonuclease fragments of virus DNA (Graham et al., 1974; van der Eb et al., 1977; Sekikawa et al., 1978; Shiroki et al., 1977) as well as studies on the effect of host-range and deletion mutants on the transforming activity of the virus (Graham et al., 1978) have located the virus genes responsible for cellular transformation to (approx.) the left-most 12% of the virus genome. This transforming region constitutes one out of five gene blocks expressed at early times after infection. RNAs transcribed from the five early regions encode at least 17 virus-specific proteins, which were characterized by several means, at least for the
non-oncogenic adenovirus types 2 and 5 (for review, see Flint & Broker, 1980). The gene products of the highly oncogenic adenovirus type 12, however, have not been sufficiently characterized so far. Neither all proteins encoded by early region E1 nor those of regions E3, E4 and E5 have been identified unambiguously. Also, less is known about the virus proteins in cells transformed with Ad12. Studies on transcription of virus genes in Ad12-transformed hamster cells have shown that, just as in Ad2- and Ad5-transformed cells, exclusively early virus genes are transcribed (Ortin et al., 1976; Smiley & Mak, 1978; Ibelgaufts et al., 1980). Moreover, in vitro translation of virus-specific RNA from Ad5- and Ad2-transformed cells has recently indicated that essentially the same virus proteins are expressed in lytically infected and transformed cells, at least from the transforming region (J. H. Lupker, personal communication; Esche, 1982).

To compare the products of the transforming genes of the non-oncogenic adenoviruses with those of the highly oncogenic adenovirus type 12, we have at first identified and characterized more precisely the Ad12-encoded proteins in infected cells. Therefore, we describe in the present report the identification and mapping of Ad12-specific proteins expressed in productively infected cells. Some of these proteins have been observed for the first time. Our analysis of the virus proteins has been based on the in vitro translation of preselected Ad12-specific RNAs prepared from the infected cells, using a cell-free system derived from rabbit reticulocytes. Applying this method we also looked for functional virus mRNAs and proteins expressed in two Ad12-transformed hamster cell lines and one Ad12-induced rat brain tumour cell line. It is shown that in comparison with the proteins found in infected cells, all three Ad12-transformed cell lines investigated express additional proteins from early regions E1 and E4.

**METHODS**

**Cell lines and virus.** Human KB cells (CCC17) were obtained from the American Type Culture Collection and were propagated in monolayer or suspension cultures in Eagle's medium supplemented with 7.5% calf serum (Seromed, Munich, F.R.G.). Human embryonic kidney (HEK) cells (obtained from Seromed) were grown in Dulbecco's medium supplemented with 10% foetal calf serum. Cell cultures were periodically screened for mycoplasma contamination (Hayflick, 1965). Human adenovirus type 12 (Ad12), a gift of W. Rowe (National Institutes of Health, Bethesda, Md., U.S.A.), was propagated in monolayer cultures of HEK cells and in spinner cultures of KB cells. The purification of the virus has been described elsewhere (Doerfler, 1969). The origins as well as the media and methods of propagation of the Ad12-transformed hamster cell lines T637, HA12/7 and the Ad12-induced rat brain tumour line RBT12/3 have been described previously (Fanning & Doerfler, 1976; Ibelgaufts et al., 1980). Some of the properties of the Ad12-transformed lines used in this study are summarized in Table 1.

**Virus DNA.** Ad12 DNA was prepared from CsCl-purified virions by SDS–Pronase B–phenol method described elsewhere (Doerfler et al., 1972). All Ad12 DNA preparations used were identified by the specific BamHI restriction endonuclease pattern of Ad12 DNA (Ortin et al., 1976).

Preparation of restriction endonuclease fragments of Ad12 DNA. The specific fragments of Ad12 DNA that we used had been cloned in the plasmids pBR322 (fragments HindIII I, F; BamHI C, B) or pBR325 (fragments EcoRI D, F and parts of fragment A) by S. Vogel, D. Eick and M. Brötz (Vogel et al., 1981). The plasmid containing the EcoRI C fragment was a gift from Dr A. J. van der Eb (Sylvius Laboratories, Leiden, The Netherlands). Plasmid DNA was isolated from Escherichia coli X1776 by slight modification of the procedure of Tanaka & Weisblum (1975).
### Table 1. Properties of the Ad12-transformed hamster and Ad12-induced rat tumour cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cells used for transformation</th>
<th>Multiplicity of Ad12 used in transformation</th>
<th>Locus of the tumour</th>
<th>T antigen</th>
<th>Oncogenicity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>T637</td>
<td>BHK21</td>
<td>350</td>
<td></td>
<td>+</td>
<td>+</td>
<td>Stroh et al. (1970)</td>
</tr>
<tr>
<td>HA12/7</td>
<td>Primary Syrian hamster</td>
<td>10</td>
<td></td>
<td>+</td>
<td>+</td>
<td>zur Hausen (1973)</td>
</tr>
<tr>
<td>RBT12/3</td>
<td></td>
<td></td>
<td>Brain</td>
<td>+</td>
<td>+</td>
<td>Ibelgaufts et al. (1980)</td>
</tr>
</tbody>
</table>

**RNA preparation.** KB cells grown in suspension cultures were infected at a density of $5 \times 10^6$ cells/ml with 20 to 30 p.f.u./cell of Ad12. After adsorption for 1.5 h, infected cells were diluted to approx. $5 \times 10^5$ cells/ml with medium supplemented with 10% calf serum and incubated at 37 °C for 9 h. When indicated, 25 μg cycloheximide or 20 μg cytosine arabinoside (araC) per ml were present from 4 h post-infection throughout infection. Cytoplasmic RNA was isolated from infected or Ad12-transformed cells as described previously (Esche et al., 1979).

**Selection and assay of virus RNA.** RNA complementary to a given region of the Ad12 genome was selected by hybridization of approx. 3000 μg cytoplasmic RNA to approx. 30 μg of appropriate fragments of Ad12 DNA immobilized on nitrocellulose filters (Ricciardi et al., 1979). Hybridization was carried out in 50% formamide, 0-01 M-PIPES pH 6.8, 0.4 M-NaCl for 3 h at 50 °C. Upon hybridization, filters were washed extensively with $1 \times$ SSC, 0.1% SDS and $0.002 \times$ EDTA, preheated to 55 °C. Ad12-specific RNA was eluted by heating the samples to 100 °C for 1 min and precipitated with 2.5 vol. ethanol at -20 °C. The RNA was then translated in a micrococcal nuclease-treated reticulocyte lysate (Pelham & Jackson, 1976). Polypeptides synthesized in the presence of $[^{35}S]$methionine (Amersham International) were analysed on 10 to 15% (w/v) polyacrylamide gels (Laemmlli, 1970) and treated for fluorography (Bonner & Laskey, 1974).

**Two-dimensional gel electrophoresis.** Isoelectric focusing was performed as described by O'Farrell (1975) and O'Farrell et al. (1977) with minor modifications. Proteins synthesized in vitro were diluted with 3 vol. 9.5 M-urea, 2% (w/v) Nonidet P40, 5% mercaptoethanol and 2% ampholines (1-4% pH range 5 to 10; 0-6% pH range 3-5 to 10) and incubated at 37 °C for 30 min. Aliquots were isoelectric focused for 4 h at 500 V (non-equilibrium pH gradient electrophoresis), or for 14 h at 400 V (equilibrium isoelectric focusing) on $11 \times 0.4$-cm cylindrical gels prepared with pH 3.5 to 10 and pH 5 to 7 ampholytes. The gels were then equilibrated in sample buffer (0.065 M-tris–HCl pH 6.8, 2.5% (w/v) SDS, 5% (w/v) mercaptoethanol, 10% (w/v) glycerol for SDS-gel electrophoresis for 30 min and applied to 15% polyacrylamide gels. After electrophoresis gels were processed for fluorography.

**Peptide maps.** Spots of $[^{35}S]$methionine-labelled polypeptides were identified after two-dimensional electrophoresis on dried gels by autoradiography and peptides were mapped essentially as described by Elder et al. (1977). The protein spots cut out from the gels were washed with 10% methanol, dried and digested with 100 μl freshly prepared trypsin in 50 mM-NH$_4$HCO$_3$ at 37 °C for 4 h, and further incubated with 0.5 ml in water overnight. The lyophilized supernatants were dissolved in 10 μl electrophoresis buffer (acetic acid:formic acid:water; 15:5:80, by vol.) spotted on a $10 \times 10$ cm cellulose-coated thin-layer plate and electrophoresed at 1000 V for 20 min. Ascending chromatography was performed in the second dimension in butanol:pyridine:acetic acid:water (32:5:25:5:20, by vol.). Plates were coated with molten 0.4% PPO in 2-methylnaphthalene (Bonner & Stedman, 1978) and fluorographed at -80 °C.
RESULTS

We have employed a translation-based assay to analyse early viral mRNAs and proteins present in cells infected and transformed with adenovirus type 12 (Ad12). Virus-specific RNA was selected from cytoplasmic RNA preparations by hybridization to virus DNA or its cloned subfragments immobilized on nitrocellulose. The mRNA eluted from the hybridization complex was then translated in a messenger-dependent reticulocyte lysate and the products identified by one- or two-dimensional gel electrophoresis followed by fluorography.

*Translation in vitro of RNAs selected with Ad12 DNA fragments representing each of the early virus regions*

Virus-specific RNA used to direct cell-free protein synthesis was prepared 9 h post-infection from KB cells with Ad12. In most experiments the cells were treated with 25 \( \mu \)g/ml cycloheximide from 2 h post-infection to harvest, because it enhances the expression of at least some early viral mRNAs (Eggerding & Raskas, 1978; Chow et al., 1979). No
Adl2 proteins in infected and transformed cells

Fig. 2. Translation in vitro of Ad12-specific E1 RNAs prepared from infected KB cells and the Adl2-transformed hamster cell line HA12/7. Messenger RNA was selected by hybridization to the Ad12 DNA fragments EcoRI C (b, f), HindIII I (c), and HindIII F (d). These experiments used RNA prepared 9 h post-infection from cells treated with 25 μg/ml cycloheximide from 2 h after infection (a to e) and from half confluent monolayers of the Ad12-transformed line HA12/7 (f). The mRNAs thus selected were translated in the reticulocyte lysate and the products analysed by electrophoresis through a 10 to 15% polyacrylamide gradient gel. Fluorography was done for 3 to 5 days. The results of cell-free translation with no added RNA (e) or RNA from infected cells selected with whole Ad12 DNA (a) are shown for comparison.

difference was found in the patterns of polypeptides produced by cell-free translation of virus RNAs isolated from cells grown in the presence or absence of cycloheximide, although RNA from cells treated with cycloheximide gave larger amounts of cell-free protein synthesis per mg of cytoplasmic RNA.

The results of in vitro translation of Ad12-specific RNAs selected with different restriction endonuclease fragments of Ad12 DNA are presented in Fig. 1 and Fig. 2. Virus RNA transcribed from early region E1 was selected with fragment EcoRI C (0 to 16.5 map units). The RNA complementary to these sequences directed the synthesis of proteins with apparent mol. wt. 59K, 38K, 36K, 30K, 28K, 26K, 25K, 24K, 22K and 19K (Fig. 1d; Fig. 2b). Several RNA preparations also directed the synthesis of a 10K polypeptide which could be separated from the labelled material running with the front by different gel systems. Some of these polypeptides, e.g. those with mol. wt. between 38K and 22K, could be documented unambiguously only by separation on polyacrylamide gradient gels as shown in Fig. 2. In addition to the proteins described above, we often detected, after longer exposures of the fluorograms, a minor polypeptide of 17K among the translation products of E1-selected RNA. Surprisingly, the RNA coding for this protein was found in much larger amounts in Ad12-transformed cells (Fig. 2f). The absence of the background bands which are seen in Fig. 1(b) in several in vitro translations also shown in Fig. 1 and Fig. 2 is probably due to
Fig. 3. Tryptic [35S]methionine-labelled peptide maps of the in vitro synthesized E1A-specific polypeptides 38K (a), 30K (b), 26K (c) and 24K (d). The protein spots, similar to those shown in Fig. 4, were digested with trypsin, spotted in the lower right-hand corner of thin-layer plates and electrophoresed from right to left in the first dimension. Peptides shown in (b) were electrophoresed for a longer time (30 min) in comparison to those shown in (a), (c) and (d) (20 min). Ascending chromatography was performed in the second dimension from bottom to top. Fluorography was done for 4 days.

differences in the different, micrococcal nuclease-treated reticulocyte S-30 extracts used. In order to determine which proteins are encoded by early region E1A and which by region E1B, RNA was selected with Ad12 DNA fragments representing only parts of region E1. Translation in vitro of RNA selected with the HindIII I fragment (6.8 to 10.5 map units), which contains exclusively E1B DNA sequences (Sawada & Fujinaga, 1980), resulted in the synthesis of proteins 59K, 19K and 17K (Fig. 1 e). This finding leads to the conclusion that these polypeptides are encoded by region E1B. The 19K and 17K proteins were also obtained with RNA selected by fragment HindIII F (10.5 to 18 map units), suggesting that its RNAs extend beyond the HindIII cleavage site at 10.5%. We still have no explanation for why we could not select with fragment HindIII I RNA, which directs the synthesis of the E1B 59K protein, although the DNA sequence coding for this polypeptide is located downstream of that coding for the 19K protein (H. Bos, personal communication). The 10K polypeptide obtained in several in vitro translations with EcoRI C-selected RNA (Fig. 1 d) was also detected with RNA selected by hybridization to fragment HindIII F but not to fragment HindIII I. This protein may represent polypeptide IX of Ad12. The polypeptides of mol. wt. between 38K and 22K were characterized as E1A-specific proteins by immunoprecipitation
Ad12 proteins in infected and transformed cells

with monospecific antibodies, directed against a synthetic peptide which is encoded by a sequence located on the 3' end of the E1A mRNA (H. Esche et al., unpublished results). To analyse further the relationship among the E1A-specific polypeptides synthesized in vitro, we compared the tryptic peptides of these proteins by high voltage electrophoresis (first dimension) combined with chromatography (second dimension). As shown for the proteins of 38K, 30K, 26K and 24K in Fig. 3, we could demonstrate major common peptides for all eight E1A polypeptides. We are currently analysing the tryptic peptides of the E1A proteins labelled in vivo as well, which we immunoprecipitated with the monospecific antibodies described above. Preliminary tryptic peptide maps of the in vitro synthesized E1B proteins 59K and 19K indicate that they are not related at all (data not shown).

RNA transcribed from early region E2 of Ad12 was selected by hybridization to fragments EcoRI F (62.2 to 64.2 map units) and BamHI C (57.9 to 73.1 map units). Translation in vitro of EcoRI F-selected RNA resulted in the synthesis of one major 61K polypeptide and some minor proteins of about 42K, 38K and 34K (Fig. 1g). RNA selected with fragment BamHI C, which comprises parts of region E3 as well as region E2, directed the synthesis of a 16K polypeptide (Fig. 1h). This protein was also obtained using RNA selected with fragment BamHI B (73.1 to 87 map units) which contains exclusively E3 DNA sequences (Fig. 1i). Therefore, we conclude that the 16K polypeptide is encoded by early region E3. RNA complementary to region E4 which was selected with the fragments EcoRI A* (approx. 87 to 100 map units) and PstI I (89.1 to 95.3 map units) was translated into proteins of mol. wt. 20K, 18K, 16K and 11.5K (Fig. 1j, k). The intensity of labelled products seen in lanes (j) and (k) is different because the polypeptides shown in lane (j) are from an autoradiogram that was deliberately over-exposed in order to detect possible additional minor polypeptides. The observation that the 16K polypeptide synthesized by PstI I-selected RNA occurred in two-dimensional gels at the same pH range (about pH 8.3) as that encoded by RNA selected with BamHI C (Fig. 4e, f) suggested that this protein is encoded by region E3. Its presence among the E4-specific translation products indicates that fragments PstI I and also EcoRI A* contain at least some E3 DNA sequences.

Analysis of Ad12-specific early proteins in two-dimensional gels

For further characterization we have analysed the virus proteins synthesized in vitro by electrophoresis on two-dimensional gels (O'Farrell, 1975; O'Farrell et al., 1977). The results illustrated in Fig. 4 confirm to a large extent our data obtained by one-dimensional gels. In addition, these results show that most E1-coded proteins as well as those of region E4 appeared at acidic pH values between 4.3 and 6.5 (Fig. 4a to c, f). The 61K protein, which probably represents the single-strand DNA-binding protein of Ad12, occurred between pH 7 and 8 (Fig. 4d, e). A similar isoelectric point was previously found for the DNA-binding protein of Ad2 (Harter & Lewis, 1978). The minor E2-specific polypeptides (45K to 34K) seen in one-dimensional gels appeared also at this pH range which led us to assume that these polypeptides represented proteolytic degradation products of the 61K protein. As mentioned in an earlier section, closer inspection of the two-dimensional gels with polypeptides
Fig. 4. Fluorograms of two-dimensional gels containing proteins synthesized in vitro by the reticulocyte cell-free system programmed with early Ad12-specific RNA. Messenger RNAs were selected by hybridization to whole Ad12 DNA (a) or the Ad12 DNA fragments EcoRI C (b, c), EcoRI F (d), BamHI C (e) and PstI I (f). RNA used in these experiments was prepared 9 h post-infection from cells grown in the presence of 25 μg/ml cycloheximide from 2 h after infection. The products of cell-free translation were analysed by two-dimensional gel electrophoresis. First dimension: non-equilibrium pH gradient isoelectric focusing (a, b, d to f) or equilibrium pH gradient isoelectric focusing (c). Second dimension: electrophoresis through 15% polyacrylamide gels. Fluorography was done for 3 to 5 days. The polypeptides are marked as follows: E2 61K (1), E1B 59K (2), E1A 38/36K (3), E1A 30/28K (4), E1A 26/25K (5), E1A 24/22K (6), E4 20K (7), E1B 19K (8), E4 18K (9), E1B 17K (11), E3 16K (12).

synthesized by RNA from region E3 and E4 (Fig. 4d, e) indicated that only one 16K protein may exist. It is encoded by region E3 and has a basic isoelectric point.

Cell-free translation of virus RNA isolated from Ad12-transformed hamster and rat brain tumour cells

In addition to RNA obtained from lytically infected cells, virus-specific RNA from two in vitro Ad12-transformed hamster cell lines (HA12/7, T637) and one Ad12-induced rat brain tumour cell line (RBT12/3) was translated in vitro. RNA was prepared from cells grown without any drugs by hybridization to the fragments EcoRI C (region E1), BamHI C (regions E2 and E3) and PstI M (region E4). E1-specific RNA prepared from each of the three Ad12-transformed cell lines directed the synthesis of proteins 59K, 38/36K, 34K, 30/28K,
26/25K, 24/22K, 19K and 17K (Fig. 2f; Fig. 5; Fig. 6c to e). The protein patterns observed resemble those which were obtained when early viral RNA prepared from infected cells was translated in vitro. However, in all Ad12-transformed cell lines investigated we found an
Fig 5. Proteins synthesized *in vitro* by the reticulocyte cell-free system programmed with virus-specific RNA prepared from the Ad12-transformed hamster cell lines HA12/7 (a) and T637 (b) and the Ad12-induced rat brain tumour line RBT12/3 (c). Messenger RNA was selected from cells grown as monolayers to half confluence by hybridization to the Ad12 DNA fragments *EcoRI* C (region E1, lane 1), *BamHI* C (regions E2 and E3, lane 2) and *PstI* M (region E4, lane 3). The products of cell-free translation were analysed by electrophoresis through 15% polyacrylamide gels which were dried and exposed for fluorography for 2 to 6 days. The results of *in vitro* translations with no added RNA or RNA selected with whole Ad12 DNA are shown for comparison (lane 4).

Fig. 6. Translation *in vitro* of E1-specific Ad12 RNA prepared from the Ad12-transformed hamster cell lines HA12/7 (c) and T637 (d), and the Ad12-induced rat brain tumour line RBT12/3 (e). RNA was prepared from cells grown as monolayers by selection/hybridization to the Ad12 DNA fragment *EcoRI* C. The products of cell-free translation of the selected RNAs were analysed by electrophoresis through 12% polyacrylamide gels followed by fluorography (3 days). Results of *in vitro* translations with no added RNA (g) and with RNA prepared from infected cells by hybridization to the fragments *EcoRI* C (a,f) and *HindIII* I (b) are shown for comparison.
Ad12 proteins in infected and transformed cells

RNA species which directed the synthesis of a 34K polypeptide not detected in Ad12-infected cells. This 34K protein shares common peptides with E1A-specific proteins (data not shown). We are just analysing the RNA coding for this protein. Region E1B RNA which codes for the 17K polypeptide was found in the Ad12-transformed cells in significantly larger amounts in comparison with cells infected with Ad12. RNA transcribed from regions E2 and E3 was detected in lines T637 and RBT12/3 only, and directed the synthesis of the 61K and 16K proteins. Unusually high amounts of E2- and E3-specific RNA were observed in the Ad12-induced rat brain tumour line RBT12/3 which synthesized in vitro additional proteins in variable amounts. The absence of any functional RNA expressed at least from region E3 in HA12/7 cells is in agreement with previous mapping data of RNA prepared from these cells (Ortin et al., 1976; Schirm & Doerfler, 1981). RNA complementary to early region E4 was detected in all Ad12-transformed cell lines investigated, and was translated into proteins of mol. wt. 25K, 24K and 20K. Some RNA preparations also directed the synthesis of an 18K and a 14.5K polypeptide. Messenger RNA for the 25K and 24K proteins, which are the most prominent bands in the fluorograms, was not found in any RNA preparation from infected cells. In general, region E4 is expressed very poorly in these Ad12-transformed cells. In none of the transformed cell lines used could mRNA for the 67K protein be detected encoded by DNA sequences between 11 and 28 map units, even upon longer exposures of the fluorograms (data not shown).

DISCUSSION

This report describes the identification and mapping of Ad12 early proteins by in vitro translation of Ad12-specific RNA prepared from infected and Ad12-transformed cells. Messenger RNA was selected from cytoplasmic RNA preparations by hybridization to different virus DNA sequences.

In the course of this study, we used RNA preparations from cells grown after infection in the presence or absence of the drug cycloheximide. Except for the observation that cycloheximide enhances the synthesis of many early RNAs (Eggerding & Raskas, 1978), we found virtually no difference in the patterns of polypeptides produced by cell-free translation of RNA from cells untreated or treated with cycloheximide.

The data from a large number of translation experiments with RNA from infected cells, similar to that of Fig. 1, are summarized in Fig. 7(a). A surprising result is that RNA specific for region E1, and particularly for region E1A, is translated into such a large number of proteins (see Fig. 1, 2 and 3): 59K, 38K, 36K, 30K, 28K, 26K, 25K, 24K, 22K, 19K and 17K. As the sum of their mol. wt. far exceeds the maximum coding capacity of region E1, these proteins cannot represent only products of separate genes. The coding area of the proteins with mol. wt. from 38K to 22K is located between 1 and 5 map units (region E1A), since their RNAs could be certainly selected with the EcoRI C fragment but not with fragment HindIII I. Peptide mapping showed that these E1A proteins are structurally related and, therefore, derived at least in part from common DNA sequences. This finding is also supported by the observation that all E1A proteins synthesized in vitro can be immunoprecipitated with monospecific antibodies directed against a synthetic peptide of 10 amino acids, which is encoded on the 3' end of E1A-specific mRNAs (H. Esche et al., unpublished results).

Recently, it has been shown by Sawada & Fujinaga (1980) that at least four RNA species with partially common sequences are transcribed from region E1A. The large number of proteins may thus be explained by the existence of overlapping RNAs as well as by premature termination and degradation of RNAs or proteins. The latter interpretation, however, seems to be unlikely, as almost all E1A proteins observed after in vitro translation of E1A-specific
RNA can be immunoprecipitated from in vivo labelled extracts of infected cells with the monospecific antibodies described above (H. Esche et al., unpublished results). A higher number of E1A polypeptides than E1A-specific RNAs was also reported for adenovirus types 2 and 5. Both viruses transcribe from early region E1A three overlapping and differently spliced RNAs (Chow et al., 1979). At least two of these RNAs each code for two related polypeptides of different mol. wt. (Esche et al., 1980). The different sizes of the two proteins derived from one RNA species might be accomplished by a post-translational modification, such as phosphorylation or proteolytic removal of a portion of at least one of the two molecules.

In vitro translation of RNA selected with fragment HindIII I resulted in the synthesis of the E1B polypeptides 59K, 19K and 17K. The finding that the E1B mRNAs coding for the 19K and 17K proteins could be selected also with fragment HindIII F indicates that the 3' ends of these RNAs map to the right of the HindIII cleavage site at 10.9 map units. These data which confirm similar observations by Jochemsen et al. (1980) would imply that transcription of early region E1B during lytic infection extends further to the right than has been reported by Ortin et al. (1976) and Smiley & Mak (1978). The fact that we failed to select mRNA which codes for the E1B 59K polypeptide, although DNA sequence data of the Ad12 E1B region indicate that the coding region for the 59K protein is located downstream of the region coding for the 19K polypeptide (H. Bos, personal communication), remains to be explained. In vitro translation of HindIII F-selected RNA, prepared early as well as late after infection in a fractionated cell-free system prepared from rabbit reticulocytes, resulted in some experiments, in addition to the proteins 19K and 17K, in the synthesis of a 10K polypeptide (data not shown). This protein which may probably be identical with the 10K polypeptide of the E1B region is not translated from E1A-specific RNAs (Esche et al., unpublished results).

Fig. 7. Adenovirus proteins synthesized in the absence of virus DNA replication. The conventional early gene blocks (E1 to E4) are shown as arrows; the direction of the arrow gives the polarity of transcription in each block. Polypeptides translated in vitro from RNA deriving from each block are listed above or below their points of origin. The circled numerals (1 to 3) mark the loci of the elements of the three-part leader associated with late mRNAs. (a) Our results; (b) results from (1) Jochemsen et al. (1980) and (2) Rosenwirth et al. (1975).
polypeptide obtained also with EcoRI C-selected mRNA (Fig. 1d) may represent polypeptide IX of Ad12.

RNA specific for early region E2a directed the synthesis of mainly one protein of mol. wt. 61K probably representing the single-strand DNA-binding protein of Ad12 (Rosenwirth et al., 1975). The weak protein bands of 45K to 34K which appeared at approx. the same pH (about 7-5) as the 61K protein in two-dimensional gels (Fig. 3) may represent proteolytic degradation of the 61K polypeptide. To early viral region E3 we could assign a protein of 16K. This protein we obtained with RNA selected among other fragments with BamHI C but also PstI I, suggesting that region E3 extends at least from 73 to 90 map units. Early region E4 codes for polypeptides of mol. wt. 20K, 18K and 11.5K. Cycloheximide strongly enhances the concentration of early region E4-specific RNAs, which is in agreement with the results of Eggerding & Raskas (1978) and Chow et al. (1979).

In addition to the proteins encoded by the conventional early regions E1, E2a, E3 and E4, we have identified a 67K polypeptide whose mRNA maps between 11 and 28 map units. It may represent the precursor of the polypeptide covalently attached to the 5' ends of Ad12 virion DNA in analogy to the Ad2 87K protein expressed from this region (Stillman et al., 1981). For comparison of our protein data with those from other groups, we have listed in Fig. 7(b) early Ad12-specific proteins which have been found by other laboratories.

Translation of Ad12-specific RNA prepared from the Ad12-transformed hamster cell lines T637 and HA12/7, as well as the Ad12-induced rat brain tumour cell line RBT12/3, showed that early regions E1 and E4 are expressed in these cell lines. We have found functional cytoplasmic RNA specific for regions E2 and E3 in lines T637 and RBT12/3 but not in the hamster line HA12/7. The expression of early region E1 in all three Ad12-transformed cell lines reflects the requirement of the products of this region for initiation and maintenance of cellular transformation. The proteins obtained with E1 RNA from the transformed cells have very similar if not identical mol. wt. as those produced by RNA from infected cells (Fig. 2, 5, 6). An additional protein of 34K, however, was found only with RNA prepared from the Ad12-transformed cells and showed common tryptic peptides with E1A polypeptides. One possible explanation for the synthesis of this 34K polypeptide by RNA from the transformed cells might be that it is encoded by an RNA species which is spliced differently in transformed cells in comparison to RNAs in infected cells. It has been shown that the processing of primary transcripts to cytoplasmic mRNAs can follow several alternative pathways with different intermediates (Chow et al., 1979). Such small variations in virus-specific RNA populations in the transformed cells may be caused by (small) differences in growth conditions of these cells. Translation of E1-specific RNA further indicated that the E1B 17K mRNA transcribed poorly in Ad12-infected cells is present in much larger amounts in the Ad12-transformed cells.

The finding that not only the transforming region but also early region E4 is expressed in all the Ad12-transformed cell lines used, which has been described for other Ad12- and Ad2-transformed cell lines also (Jochemsen et al., 1980; H. Esche, unpublished results), raises the question as to whether the function of some of the E4 gene products can exert any influence on the transforming process or the phenotype of the transformed cells. The protein patterns observed with E4-specific RNA prepared from the transformed cells showed, in addition to proteins obtained with RNA from infected cells, two proteins of 25K and 24K. We are currently analysing these proteins in more detail.

RNAs transcribed from early regions E2 and E3 in cell lines T637 and RBT12/3 directed mainly the synthesis of the polypeptides found using RNA from infected cells (E2 61K, E3 16K), which are apparently not required for transformation. That we have not obtained E3-specific proteins with RNA from the Ad12-transformed hamster line HA12/7 confirms the observation by Ortin et al. (1976) and Schirm & Doerfler (1981) that region E3 is not
transcribed in these cells. RNA from region E2, however, was shown to be expressed and transported into the cytoplasm of HA12/7 cells (Ortin et al., 1976). Why this RNA cannot be translated in vitro has still to be explained.

In none of the transformed cell lines investigated has virus RNA been found transcribed from DNA sequences between 11 and 28 map units. Functional RNA from late viral regions has not been detected either (Schirm & Doerfler, 1981).

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REFERENCES


Adl2 proteins in infected and transformed cells


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