Characterization of the 55K adenovirus type 5 E1B product and related proteins

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In addition to major proteins of 19K and 55K (176 and 496 residues, 176R and 496R, respectively), early region 1B (E1B) of human adenovirus type 5 (Ad5) is predicted to encode at least three other polypeptides of 156R, 93R and 84R that share 79 amino-terminal residues with 496R. We have used a series of specific antipeptide sera to identify and partially characterize these proteins. 84R was produced in large amounts, 156R somewhat less, and 93R at very low levels. Synthesis of 176R, 496R, as well as the E2A 72K DNA-binding protein commenced shortly after that of E1A proteins in Ad5-infected KB cells. Production of 156R, 93R and 84R began somewhat later, but prior to the synthesis of the late structural protein IX and hexon. 156R, which is composed of the 79 amino-terminal and 77 carboxy-terminal amino acids of 496R, migrated on SDS-PAGE as two species which appeared to differ by their degree of phosphorylation. 156R and 496R yielded identical tryptic phosphopeptides that contained both phosphoserine and phosphothreonine, and one of these was immunoprecipitated by a serum specific for the carboxy terminus. These results suggested that Ser-490 and/or Ser-491 as well as Thr-495 are major sites of phosphorylation in these proteins.

Introduction

Products of the early region 1B (E1B) of human adenoviruses (Ad) are involved in both virus replication and cell transformation (Branton et al., 1985a and references therein and below). The 2-2 kb E1B early mRNA of serotype 2 (Ad2) produces two major E1B proteins, a 19K polypeptide of 175 residues (175R) and, from an internal initiation site and alternative reading frame, a 55K 495R product (Perricaudet et al., 1979; Bos et al., 1981; Gingeras et al., 1982). A second major E1B mRNA of 1-0 kb accumulates later during infection and encodes both 175R and an 82R protein possessing an amino terminus identical to that of 495R. Minor E1B mRNAs of 1-26 and 1-31 kb are also transcribed at later times and both produce 175R (Virtanen & Pettersson, 1985; Anderson et al., 1984; Lewis & Anderson, 1987). The former, from the 495R start site, also encodes a 155R protein with amino and carboxy termini identical to those of 495R (Green et al., 1982; Anderson et al., 1984; Lucher et al., 1984). The latter is believed to produce a 92R polypeptide with the same 495R-related amino terminus but a unique carboxy terminus. Serotype 5 (Ad5), which is very similar in DNA sequence to Ad2, is predicted to encode almost identical E1B proteins (see Fig. 1) of 496R, 176R, 156R, 93R and 84R (Perricaudet et al., 1979; Bos et al., 1981). Another E1B mRNA of 0-86 kb has also been proposed (Lewis & Anderson, 1987). As illustrated in Fig. 1, in Ad5 this mRNA would produce both a 131R protein composed of the 126 amino-terminal residues of 176R linked to the five amino acid carboxy-terminal region found in 84R, and a 168R product made up of the first 26 residues of 496R followed by a short polyglycine stretch and then the complete coding sequence of late protein IX. These products have not been identified.

Much of the transforming potential of adenoviruses derives from products of early region 1A (E1A) which can immortalize cells and induce abortive transformation. However, generation of stable transformants of primary rodent cells requires E1B proteins. The 19K and 55K E1B proteins can function independently, but transforming efficiency is increased when both are expressed (Bernards et al., 1986; Barker & Berk, 1987; White & Cipriani, 1990; Yew et al., 1990; McLorie et al., 1991; Zhang et al., 1992). The 19K protein appears to protect against programmed cell death induced as a consequence of disruption of growth control pathways by E1A (White et al., 1992; Rao et al., 1992). The 55K protein may derive some or all of its transforming activity from interactions with and inactivation of the cellular p53 tumour suppressor (Sarnow et al., 1982b; Kao et al., 1990; Yew & Berk, 1992). This E1B protein is also required for productive infection of human cells and functions in the accumulation of late viral mRNAs, control of late viral protein synthesis, and shutoff of host...
cell metabolism (Babiss & Ginsberg, 1984; Logan et al., 1984; Babiss et al., 1985; Halbert et al., 1985; Pilder et al., 1986; Bernard et al., 1986; Barker & Berk, 1987; Sandler & Ketner, 1989; Bridge & Ketner, 1990; Leppard & Shenk, 1989; Yew et al., 1990; McLorie et al., 1991). With serotype 12 (Ad12), the corresponding 54K protein is required for viral DNA replication (Shiroki et al., 1986; Breiding et al., 1988; Mak & Mak, 1990; Zhang et al., 1993). The roles of the other 496R-related proteins in both virus replication and cell transformation are unclear. Studies involving mutants designed to eliminate the synthesis of one or more of these proteins suggested that they were of little functional importance (Logan et al., 1984; Montell et al., 1984). However, Lewis & Anderson (1987) have suggested that they could act to modulate expression of different domains of 496R.

In the present experiments we have studied Ad5 E1B products using a series of specific anti-peptide sera. In addition to identifying the various E1B proteins and studying their synthesis, we also found that phosphorylation of the 496R 55K polypeptide and 156R occurs largely at serine and threonine sites at the carboxy terminus.

Methods

Cells and viruses. Human KB cells were cultured on 100 mm (diameter) dishes (Corning Glass Works) in x-minimal essential medium supplemented with 10% fetal calf serum and were infected with mutant or wild-type (wt) Ad5 at an m.o.i. of 35 p.f.u./cell, as described previously (Rowe et al., 1983). The strain used as wt was dl309 (Jones & Shenk, 1979). Plaque assays were performed on human KB cells or Ad5-transformed 293 cells (Graham et al., 1977).

Antisera and immunoprecipitation. Antisera to the carboxy termini of 176R and 496R, termed 19-C1 and 58-C1, respectively, have been described previously (McGlade et al., 1987; Yee et al., 1983). Antisera to the amino termini of 496R, 84R, 93R and 156R, termed 58-N1, and the carboxy termini of 84R and 93R (84-C1 and 93-C1, respectively) were prepared against the synthetic peptides indicated in Table 1 (Yee et al., 1983). Cell extracts were immunoprecipitated as described previously (McGlade et al., 1989) using 25 µl of antipeptide sera. E1A proteins and the E2A 72K polypeptide were precipitated using mouse monoclonal antibodies M73 (Harlow & Lane, 1988) and H2-67 (Branton et al., 1985), respectively, and late structural protein IX by a polyclonal rabbit serum prepared against purified protein (a gift of W. C. Russell).

Radioactive labelling. Ad5- or mock-infected cells were labelled from 16 to 18 h post-infection with 100 µCi of [35S]methionine (Amersham; specific activity 1300 Ci/mmol) or 2.5 mCi of [35S]methionine- or phosphate-free medium.

SDS-PAGE. Immunoprecipitates were analysed by SDS-PAGE using 15% polyacrylamide separating gels, as previously described (Rowe et al., 1983).

Analysis of tryptic peptides by HPLC. [35S]Methionine-labelled E1B proteins purified by immunoprecipitation and SDS-PAGE were extracted and treated with TPCK-trypsin (Worthington), as described previously (Tremblay et al., 1988, 1989). Tryptic peptides were separated on a Waters dual pump HPLC system with a 600E controller (Waters Associates). Peptides were eluted from the column by a linear gradient of solution A (5% formic acid in water) to solution B (5% formic acid in ethanol) for 95 min at a flow rate of 1 ml/min, as described previously (Dumont & Branton, 1992). Detection of labelled peptides was achieved using an on-line LB507A isotope detector (Berthold).

Phosphoamino acid analysis by TLC. Proteins were subjected to acid hydrolysis and after mixing with non-radioactive phosphoamino acid markers the material was subjected to electrophoresis at pH 3.5 in

Fig. 1. Predicted Ad5 E1B products and amino- and carboxy-terminal sequences. (a) The Ad5 E1B mRNAs and protein products predicted from DNA sequences and mapping studies (see text) have been illustrated. Included also are relevant nucleotides for translation start and stop sites and for splice donors and acceptors. (b) Amino and carboxy termini of 496R which form the 156R protein The residue numbers have been presented above the sequence and the positions of cleavage by trypsin and the tryptic peptide numbers below. Numbering was based on amino acids of the 496R protein.
Table 1. Antipeptide sera specific for Ad5 E1B products

<table>
<thead>
<tr>
<th>Peptide serum</th>
<th>Amino acid sequence</th>
<th>Proteins predicted to be recognized</th>
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<tbody>
<tr>
<td>176R C terminus</td>
<td>NH₂... RAGLDPR-E-COOH</td>
<td>176R</td>
</tr>
<tr>
<td>496R/156R C terminus</td>
<td>NH₂... SDEDTD-E-COOH</td>
<td>496R, 156R</td>
</tr>
<tr>
<td>496R N terminus</td>
<td>NH₂-MERRNP-EG...-COOH</td>
<td>496R, 156R, 84R, 156R (168R?)</td>
</tr>
<tr>
<td>84R C terminus</td>
<td>NH₂... GMNVVQPPP-PP-COOH</td>
<td>84R, 496R</td>
</tr>
<tr>
<td>84-C₁ peptide:</td>
<td>NH₂-YGMNVVQQPPP-COOH</td>
<td>84R</td>
</tr>
<tr>
<td>84-C₂ peptide:</td>
<td>NH₂-YVQPPP-COOH</td>
<td>84R</td>
</tr>
<tr>
<td>93R C terminus</td>
<td>NH₂... PTLPMQFESH-COOH</td>
<td>93R</td>
</tr>
<tr>
<td>93-C₁ peptide:</td>
<td>NH₂-YPTLPMQFESH-COOH</td>
<td></td>
</tr>
</tbody>
</table>

* Synthetic peptides were purchased from Biosearch or Bachem and coupled to carrier BSA via tyrosine residues, as described previously (Yee et al., 1983). Antisera were raised in New Zealand rabbits. Sequences of the termini of E1B proteins and the synthetic peptides have been indicated. The methionine residue in the 58-N₁ peptide was acetylated (Ac).

Results

Identification of 496R-related proteins using antipeptide sera

Antipeptide sera have been used to identify the Ad5 176R and 496R E1B proteins (and 175R and 495R of Ad2) as polypeptides that migrate in SDS-PAGE with apparent $M_r$ values of 19,000 and 55,000, respectively (Yee et al., 1983; Anderson et al., 1984; McGlade et al., 1987; Lewis & Anderson, 1987; McLorie et al., 1991). Ad5 E1B is predicted to encode at least three additional proteins of 84R, 93R and 156R with amino termini identical to that of 496R. The 84R and 93R species have unique carboxy termini whereas those of 156R and 496R are identical. To identify these Ad5 proteins, antipeptide sera were prepared against peptides corresponding to the predicted termini of these species (see Table 1). Ad5-infected cells were labelled with $[^{35}S]$methionine and extracts immunoprecipitated using various antipeptide sera, in some cases in the presence of the peptide against which the sera were raised, and then analysed by SDS-PAGE. Fig. 2 and 3 show the results of several experiments. As found previously (Yee et al., 1983), 58-C₁ serum, which recognizes the carboxy terminus of 496R, precipitated this 55K species (Fig. 3, lane 7). Serum 58-N₁ which was prepared against the amino terminus of 496R also precipitated this same species (Fig. 2, lane 1; Fig. 3, lane 6), and in addition recognized several other proteins, including a major species of about 20K, two polypeptides of about 25K, and several other minor species between 21K and 30K. As discussed in detail below, results from $^{32}P$ labelling experiments suggested that at least four such minor proteins were present to which, for ease of discussion, we have attributed approximate $M_r$ values of 21K, 21.5K, 22K and 30K. Addition of the 58-N₁ peptide clearly blocked precipitation of 496R and most of these other species (Fig. 2, lane 2), indicating that all were specifically recognized by the 58-N₁ serum. A possible exception was the 30K protein for which detection was only partially diminished by addition of competing peptide. None of these proteins was precipitated from mock-infected cells using 58-N₁ serum (Fig. 3, lane 12) or from infected cells using 19-C₁ serum which recognizes the carboxy terminus of the 19K (176R) protein (McGlade et al., 1987).

To study these polypeptides further, sera were raised against peptides corresponding to the predicted unique carboxy terminus of 84R and 93R. Two 84R-specific sera were prepared (see Table 1). Serum 84-C₁ was produced using a peptide containing the entire five-residue carboxy terminus unique to 84R plus six residues also present in 496R (amino acids 74 to 79 within the region common to both E1B products). The pattern of precipitation by this serum was similar to that obtained with 58-N₁ (data not shown). It was therefore likely that antibodies were generated that recognized an epitope defined by the six common residues, making 84-C₁ serum of little use in identifying E1B products. For this reason, a second antiserum, 84-C₂, was produced against a smaller peptide that contained just two such common residues (see Table
Fig. 2. Identification of Ad5 E1B products using antipeptide sera. Ad5-
and mock-infected KB cells were labelled with [35S]methionine or 32P,
from 16 to 18 h p.i. and cell extracts were immunoprecipitated with
E1B-specific antipeptide sera, in some cases in the presence of 10 gg of
the corresponding peptide, and precipitates were analysed by SDS-
PAGE. The contents of each lane have been indicated in the figure. The
positions of 496R, 156R, 93R, 84R and the 21K, 22K and 30K proteins
have been indicated on the right and those of 14C-labelled Mr markers
on the left.

1). This serum recognized only the 20K species (Fig. 2,
lane 5; with competing peptide lane 6) and this
polypeptide was not precipitated by 58-C1 serum (Fig. 3,
lane 7). These results suggested that this protein
represented 84R which was clearly produced at high
levels in infected KB cells. Serum 93-C1, against the
predicted carboxy terminus of 93R, precipitated a minor
species of about 21.5K that probably represented this
Ad5 E1B product (Fig. 2, lane 9; with competing peptide
lane 10). In addition, particularly with the use of larger
amounts of this serum (Fig. 3, lane 9), the 22K species
was also detected. As discussed below, this latter
polypeptide could represent a modified form of 93R. The
156R E1B product was also tentatively identified. The
two species migrating at about 25K were both precipi-
tated by 58-C1 and 58-N1 sera (Fig. 3, lanes 7 and 6,
respectively), as predicted for 156R. These results
indicated that two proteins with structures generally
similar to 156R must exist. The identities of the 21K and
22K species precipitated by 84-C2 and 58-N1 will be
discussed further below.

The 168R species proposed by Lewis & Anderson
(1987) is predicted to contain the amino terminus of
496R followed by the entire sequence of late protein IX.
Extracts from infected cells were precipitated using
polyvalent serum prepared against protein IX and Fig. 3
(lane 10) shows that the major 14K protein IX
polypeptide was detected, as was a minor species of
about 38K. This species was also recognized by the 58-
N1 serum (Fig. 3, lane 6), but precipitation was only
partially blocked by addition of competing peptide (Fig.
2, lanes 1 and 2). Thus it was possible, but not certain,
that this protein represented 168R.

Analysis of tryptic peptides
To provide further evidence for the identity of these E1B
species, tryptic peptides prepared from proteins labelled
with [35S]methionine were analysed by HPLC using
reverse-phase columns. 496R should yield 52 tryptic
peptides of which 10 should contain methionine. Fig.
4(a) indicates that about 10 major 35S-labelled peptides
(A to K) were evident for 496R. Additional species could
also result from incomplete trypsin digestion. A similar
analysis of the 20K protein recognized by 58-N1 and 84-
C2 sera revealed two labelled species (Fig. 4b). The 84R
protein should produce two methionine-containing tryp-
tic peptides, one identical to the amino-terminal peptide
of 496R, the other unique. One of the labelled peptides
coloured with peptide B of 496R. The second, termed E-
1, eluted in a position intermediate between peptides E
and F of 496R. These results confirmed the identity of
the 20K species as 84R. The slower and faster migrating
25K species recognized by 58-N1 and 58-C1 sera were
analysed separately, and found to produce similar trypsin
peptide patterns (Fig. 4c, d, respectively). Both yielded
peptides that eluted with properties similar to peptides B,
G and I (and possibly K) of 496R, and an additional
unique peptide (D-1) that eluted in a position inter-
termediate between peptides D and E of 496R. One or
more additional species was also evident in both. The
156R protein should yield four methionine-containing
peptides, three identical to those present in 496R and one
unique. These results indicated that both proteins were
highly related and probably represented two forms of
156R (see further below). We were unsuccessful in
obtaining sufficient labelled material to confirm positively that the 21.5K protein precipitated by 93-C1 serum was 93R. However, based on the specificities of the sera employed, it was highly likely that such was the case. Similarly, the 30K protein could not be identified by this method.

**Time-course of synthesis of Ad5 proteins**

To determine the time-course of synthesis of 496R-related proteins, virus-infected cells were labelled with [35S]methionine for 2 h intervals from 0 to 20 h p.i. Cell extracts were divided into aliquots which were immunoprecipitated with various antisera and the proteins were analysed by SDS-PAGE. Fig. 5 shows that synthesis of E1A products occurred immediately, between 0 and 4 h, and was maximal between 4 and 10 h. Slightly later (4 to 6 h p.i.), synthesis of E1B, 176R, 496R and the early region 2A (E2A) 72K ssDNA-binding protein commenced and continued at high levels throughout the infection. Synthesis of 156R and 93R commenced after 6 h and rose steadily throughout the period examined, whereas that of 84R began between 8 to 10 h. The Ad5 structural proteins IX, hexon, penton base, penton-associated protein and fibre (Horwitz, 1990) were undetectable until shortly before 10 h p.i., after which synthesis proceeded at high levels. As discussed below, synthesis of these proteins was in general agreement with the time of appearance of the mRNAs of the appropriate E1B mRNAs.

**Synthesis of E1B proteins in Ad5-transformed 293 cells**

The pattern of E1B protein synthesis was also determined in Ad5-transformed human 293 cells which contain the entire E1A and E1B regions of Ad5 (Graham et al., 1977). Cells were labelled with [35S]methionine and extracts were precipitated using various antisera. Fig. 3 (lane 1) shows that multiple species of E1A proteins were produced at about one-fifth to one-tenth the maximum level found in infected KB cells (data not shown). In addition to 496R, 58-N1 serum precipitated low levels...
Phosphorylation of 496R-related proteins

The 496R protein is known to be phosphorylated at multiple sites (Sarnow et al., 1982a; Yee et al., 1983; Malette et al., 1983). To determine whether any of the 496R-related proteins are phosphorylated, Ad5-infected cells were labelled with $^{32}$P, and E1B proteins were precipitated using antipeptide sera. Fig. 2 shows that 496R was clearly phosphorylated (lane 3), but no $^{32}$P-labelled species were observed in the positions of the 20K (84R) protein (see lanes 3 and 7) or the 21.5K (93R) species (lane 11). In the case of 156R, the slower migrating form was clearly labelled to a much greater extent than was the faster migrating species (lane 3). Thus the difference in gel migration of these two 156R forms appeared to be due to differences in phosphorylation. Similar shifts in gel mobility caused by differential phosphorylation have been observed with Ad5 E1A proteins (Dumont et al., 1989). In addition to these proteins, two other phosphorylated species were observed that migrated to the positions of the 21K and 22K species described above. These phosphoproteins both appeared to be recognized by 58-N1 (lane 3) and 84-C2 (lane 7) sera, suggesting that both could represent minor phosphorylated forms of 84R. Results with 93-C1 serum (lane 11) were inconclusive regarding the existence of phosphorylated forms of 93R (see Discussion).

Identification of major phosphorylation sites in 496R and 156R

That 156R is phosphorylated indicated that common phosphorylation sites may exist in 496R. To analyse such phosphorylation sites further, Ad5-infected cells were labelled with $^{32}$P, and following immunoprecipitation with 58-N1 serum and separation by SDS–PAGE, 496R and 156R were isolated, digested with trypsin, and tryptic phosphopeptides were analysed by reverse-phase chromatography using HPLC. Fig. 6(a) indicates that

![Fig. 4](image-url)
three labelled species were produced from 496R. Species I was found by TLC to migrate in the position of free phosphate (data not shown). Thus 496R contained only two closely eluting phosphopeptides, termed species II and III. Fig. 6(b) shows that, in addition to free phosphate, 156R contained two phosphopeptides that eluted exactly as did peptides II and III. These data suggested that the major sites of phosphorylation of 496R must be present within the first 79 or last 77 residues of 496R that form the 156R protein. Fig. 1(b) shows that many potential serine and threonine sites exist within these regions. However, it was unlikely that such sites existed within the amino-terminal portion because all potential sites, except for Ser-7, are present within the large tryptic peptide T4 which, in 156R, is fused to peptide T43 to form a unique peptide not present in 496R. In addition, 84R, which shares the same amino terminus, was found not to be highly phosphorylated (Fig. 2, lanes 5 and 7). Although several potential serine and threonine sites existed within the common carboxy terminal 77 residues, likely candidates were Ser-490, Ser-491 and Thr-495 which are present in the carboxy-terminal peptide T52 within sequences characteristic of substrates of casein kinase II or I (Pearson & Kemp, 1991). To determine whether peptide T52 contained phosphorylation sites, 32P-labelled 156R was digested with trypsin and the mixture was then immunoprecipitated with 58-C1 serum prepared against a carboxy-terminal peptide. Fig. 6(c) shows that a phosphopeptide which eluted somewhat later than the synthetic...
peptide to which 58-C1 serum was prepared (see arrow), but with characteristics of peptide II, was evident upon analysis by HPLC. Peptide II was isolated, submitted to acid hydrolysis, and phosphoamino acids were separated by TLC. The results indicated that both phosphoserine and phosphothreonine were present (data not shown). These results indicated that peptide II must represent tryptic peptide T52 and that the major sites of phosphorylation of 156R and 496R are located within peptide T52, presumably at Thr-495 and Ser-490 and/or Ser-491.

Discussion

In the present study we have used a series of antipeptide sera with specificity for predicted amino and carboxy termini to identify and partially characterize Ad5 E1B products. In addition to 176R and 496R which have been extensively studied, we showed that Ad5 E1B produces several other polypeptides which have amino termini identical to that of 496R and which migrate in SDS–PAGE with apparent $M_r$ values of 20000 to 30000. Gel migration of these species was slower than expected from their predicted sizes which range from 8K to 16.5K (Virtanen & Pettersson, 1985). The 84R protein, as was the case with the equivalent 82R product of Ad2 (Lewis & Anderson, 1987), was produced in large quantities particularly late during infection. Two 156R species were identified that were synthesized earlier after infection than 84R. The 93R product of the 1.31 kb mRNA was also identified for the first time and found to be produced in extremely small quantities. Earlier studies on E1B expression (Virtanen & Pettersson, 1985) indicated that of the major E1B transcripts, the 2.2 kb mRNA (encoding 176R and 496R) is preferentially expressed at early times whereas the 1.0 kb mRNA (176R and 84R) accumulates late after infection. The minor 1.26 (176R and 156R) and 1.31 (176R and 93R) kb mRNAs are expressed both early and late; however, the former is in excess by 20-fold. Our observations on E1B protein synthesis were in complete accord with these data.

It is possible that the two 156R-related species are produced from similar but alternatively spliced mRNAs, but the simplest explanation was that these species were generated by differences in phosphorylation. It is likely that the major phosphorylation sites for 156R reside within the carboxy-terminal 77 residues as neither 84R nor 93R, which share with 156R identical 79 residue amino termini, appeared to be highly phosphorylated (but see below). The production of two $^{32}$P-labelled tryptic peptides (II and III) indicated that two or more sites must exist within this region. 58-C1 serum, which is specific for the carboxy termini of 496R and 156R, appeared to recognize only peptide II. Thus either only peptide II corresponded to tryptic peptide T52, or both peptides II and III originated from T52, but III was not efficiently recognized by the 58-C1 serum. Detection of both phosphoserine and phosphothreonine in peptide II indicated that multiple phosphorylation sites must exist within T52, and thus peptides II and III could represent differentially phosphorylated forms of T52 which were not recognized equally by 58-C1. In addition, species II and III could result from incomplete trypsin digestion which yielded both peptide T52 and a partial product containing both T51 and T52. 58-C1 serum may not recognize both of these forms equally. Two threonine residues are present in T51 at positions 471 and 484, but neither is present within obvious protein kinase consensus sequences. Peptide T52 contains two serine residues (Ser-490 and -491) that exist within a sequence characteristic of substrates of casein kinase II (Pearson & Kemp, 1991). One or both of these sites could be phosphorylated. Thr-495 exists within an ideal casein kinase I sequence and thus could account for the phosphothreonine present in 496R and 156R. Thus, although it is likely that Ser-490, Ser-491 and Thr-495 are phosphorylated, confirmation of these sites and assessment of biological relevance will await generation of appropriate mutants.

In addition to the two forms of 156R, 58-N1 serum recognized 21K and 22K phosphoproteins which were also detected using 84-C2 serum. These could represent additional E1B products. However, we believe it more likely that they are minor phosphorylated forms of 84R which, as in the case of 156R, migrate slightly slower than the unphosphorylated or underphosphorylated species. Several sites exist within the amino-terminal 79 residues of 496R that could serve as substrates for casein kinase II or members of the p34$^{dor}$ or MAP (mitogen-activated protein) kinase families (Pearson & Kemp, 1991). An additional 30K species was also observed, and because it was recognized by serum against protein IX and possibly 58-N1, it probably represents the 168R protein predicted by Lewis & Anderson (1987). Further studies involving both peptide mapping and analysis of mRNA will be required to confirm this possibility.

The roles of these other E1B proteins are unclear but they could function in virus replication and perhaps play some auxiliary role in the establishment or maintenance of transformed cells. Ad5-transformed 293 cells were found to express all proteins encoded within the E1B region including 176R, 496R, 84R, 93R, 156R, and even late protein IX. Several of these products have been found to be produced in normal lytic infection only at later times. 496R appears to derive transforming activity through interactions with the cellular tumour suppressor p53 (Sarnow et al., 1982b; Kao et al., 1990; Yew & Berk, 1992). It is possible that 156R, 84R or 93R could function in transformation or replication by interfering
with or enhancing this interaction or some other function of 496R. Other approaches will be necessary to investigate the importance of these proteins further.

Note added in proof. We have now shown that Ser-490 and/or Ser-491 are phosphorylation sites present in tryptic phosphopeptides II and III from 496R and 156R, and obtained indirect evidence that Thr-495 is probably also phosphorylated [Tedoro et al. (1994) Journal of Virology 68, 776–786].

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References


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