The Kinetics of Synthesis of Early Viral Proteins in KB Cells Infected with Wild-type and Transformation-defective Host-range Mutants of Human Adenovirus Type 5

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SUMMARY

We have studied the kinetics of early adenovirus type 5 (Ad5) protein synthesis during lytic infection of KB cells by wild-type (wt) and transformation-defective host-range (hr) mutants. Proteins encoded within four early regions were studied: early region 1A (E1A: 1.5 to 4.5 map units, mu), E1B (4.5 to 11.2 mu), E2A (61.6 to 74.9 mu), and E4 (91.4 to 99.1 mu). Synthesis of E1A products, the first to appear during wt lytic infection, was detectable within 2 h after injection, reached a peak within the next hour, then declined to very low levels by 7 h post-infection. Synthesis of E2 and E4 proteins began at about 3 h post-infection, was maximal by 6 h and thereafter declined sharply. The E1B 19K and 58K proteins were first detected around 3 h post-infection and, after reaching maximal levels of expression by 8 h, declined to lower levels by 12 h post-infection. Infections with the E1A mutant hr3 were characterized by greatly depressed levels of early expression of E1B, E2 and E4 polypeptides but protein synthesis from these regions appeared to recover at late times. The pattern of expression exhibited by the E1B mutant hr6 revealed delayed and reduced levels of expression of E1B, E2 and E4 protein synthesis but increased levels of E1A protein synthesis. These results are consistent with the reported role of E1A gene products in the activation of early gene expression and, in addition, suggest that a function encoded in E1B may also influence the expression of Ad5 early genes at early and late times.

INTRODUCTION

Expression of the adenovirus genome in productively infected cells is temporally divided into two distinct phases, early and late, corresponding to events occurring before or after the onset of viral DNA replication. During the early phase only a portion of the genome, consisting of five discrete regions and involving both strands of the viral DNA, is expressed (Flint, 1977; Galos et al., 1979). Early nuclear RNA is transcribed from the 'r' strand of early region 1 (E1: 1.5 to 11.2 map units, mu), from the 'l' strand of early region 2 (E2A: 61.6 to 74.9; E2B: 11.2 to 23.5), from the 'r' strand of early region 3 (E3: 76.8 to 86.0), and from the 'l' strand of early region 4 (E4: 91.4 to 99.1) (Berk & Sharp, 1978; Chow et al., 1979; Stillman et al., 1981; Gingeras et al., 1982). Small amounts of RNA from the 'r' strand of the region 16.5 to 40.0 (L1), representing a portion of the sequences expressed from the major late promoter operating after DNA replication, have also been detected at early times (Lewis & Mathews, 1980; Shaw & Ziff, 1980).

Several lines of evidence have implicated viral early gene products as regulators of gene expression. S1 nuclease gel analysis of steady-state levels of viral RNA in cells infected with host-range (hr) mutant hr1 (which maps in E1A; Galos et al., 1980) has implicated one or more E1A products as a positive effector in the synthesis of stable nuclear RNA from E1B, E2, E3 and E4 regions (Berk et al., 1979). The 13S and 12S mRNAs complementary to this region are processed from alternative 5' splice sites (5'SS) to a common 3' splice site (3'SS) of the same initial transcript and are translated into polypeptides detected both in vitro and in vivo which...
share common amino and carboxy peptides but differ in the length of the internal amino acid sequences (Perricaudet et al., 1979; Halbert et al., 1979; Esche et al., 1980; Smart et al., 1981). The smallest of the E1A messages, a 9S mRNA, has recently been shown to splice into an alternative 3' reading frame and thus has only amino terminal peptides in common with 12S and 13S RNAs (Svensson et al., 1983). The hr1 mutant carries a single base deletion in sequences that are unique to the 13S mRNA and thus synthesizes a truncated 13S product but, unless hr1 carries additional mutations, normal 12S and 9S products (Ricciardi et al., 1981).

Recent studies on the kinetics of RNA synthesis in E1A mutant-infected cells show that early transcription is repressed in dl 312 [a deletion mutant expressing no E1A proteins (Jones & Shenk, 1979b)] or in hr1 infections (Nevins, 1981) and that to a degree this repression can be overcome by protein synthesis inhibitors. To date the studies with mutants of E1A and drug-treated cells have identified a requirement for an E1A gene product as an activator of early gene expression and raised the possibility of further involvement of E1A products in stabilization and translation of early mRNA (Berk et al., 1979; Nevins et al., 1979; Jones & Shenk, 1979a; Persson et al., 1981; Katze et al., 1981; Nevins, 1981; Solnick, 1981; Solnick & Anderson, 1982; Shaw & Ziff, 1982).

Studies of early transcription with the adenovirus type 5 (Ad5) E2A mutant, ts125, which produces a temperature-sensitive DNA-binding protein (DBP), have shown that this product specifically represses transcription of region E4 (Nevins & Winkler, 1980). In addition, the cytoplasmic stabilities of at least E1 mRNAs have been shown to be three to five times greater in ts125-infected cells than in wild-type (wt)-infected cells at the non-permissive temperature, implying a role for the DBP not only as a repressor of E4 but also as a destabilizer of early adenovirus RNA (Babich & Nevins, 1981).

The potential involvement of E1B in the regulation of early gene expression has also been explored (Berk & Sharp, 1978; Ross et al., 1980b), but the results have not revealed as clear a role for E1B products as has been found for E1A and E2A. E1B encodes 13S and 22S mRNAs spliced from alternative 5'SS to a common 3'SS and an independently promoted unspliced 9S mRNA. Both 13S and 22S direct the synthesis of the 19K major tumour antigen but the 22S also directs the synthesis of the 58K major tumour antigen by utilizing an internal translation initiation site for a different reading frame which partially overlaps the coding sequence for the 19K antigen (Bos et al., 1981). The unspliced 9S mRNA contains a single open reading frame encoding the virion structural polypeptide IX (Alestrom et al., 1980). The steady-state levels of early cytoplasmic mRNA synthesized in HeLa cells infected with the E1B mutant hr7 are not significantly different from those of wt-infected cells as determined by S1 nuclease gel analysis (Berk et al., 1979).

Comparison of levels of DBP in wt- and hr7-infected cells pulse-labelled for 4 h at 12 h post-infection led Ross et al. (1980b) to the conclusion that this product was overproduced in hr7 infections and suggested that region E1B may encode a negative regulator of adenovirus early gene expression. Lassam et al. (1978) reported that similar levels of DBP were synthesized by wt and hr6 (an E1B mutant phenotypically similar to hr7; Harrison et al., 1977) infected cells in 1 h pulses at 24 h but the level of late gene expression in hr6-infected cells was reduced compared to wt. Both hr6 and hr7 (mapping between 6-0 and 8-5 mu; Galos et al., 1980) exhibit a multiplicity-dependent leakiness for virus production but synthesize no 58K tumour antigen detectable by immunoprecipitation and polyacrylamide gel electrophoresis (Lassam et al., 1979; Ross et al., 1980b) even at very high (100 p.f.u./cell) multiplicities.

Most of the studies described above have examined either steady-state levels or rates of RNA synthesis or have utilized limited pulse-labelling analysis of protein synthesis in wt- and mutant-infected cells. Only Neuwald et al. (1977) have provided data on the time course of early protein synthesis in infected KB cells. Although the coding regions of the early proteins examined (72K, 19K and 11K) were not precisely identified, it is now clear that the 72K protein was the E2 DBP (Levine et al., 1974; Ginsberg et al., 1977), the 19K protein was the E3 membrane glycoprotein (Persson et al., 1979, 1980) and the 11K protein was an E4 nuclear protein (Chin & Maizel, 1977; Sarrow et al., 1982; Downey et al., 1983). Each protein showed unique kinetics of expression which were in approximate agreement with the subsequently determined level of transcriptional
activity for the corresponding early regions (Nevins et al., 1979). In the present study, we have examined the kinetics of expression of E1, E2 and E4 at the level of protein synthesis in infected KB cells and the effect of mutations in E1A and E1B on early gene expression. We show that expression of E1B as well as E1A is involved in the regulation of early gene expression at early times post-infection.

METHODS

Cells and virus. KB cells were cultured as monolayers on 150 mm diam. dishes (Lux) in α-minimal essential medium (α-MEM) (Stanners et al., 1971) supplemented with 10% horse serum (HS). For preparation of spinner cultures, the cells were suspended (in Joklik's modified medium supplemented with 10% HS) at densities between 4 × 10^6/ml and 1 × 10^7/ml and grown to a density of 3 × 10^5/ml in Bellco spinner culture flasks. The 293 strain cells used for the propagation of hr mutants have been described previously (Graham et al., 1977). These cells were grown as monolayers in Joklik's modified medium supplemented with 10% HS.

The wt strain of Ad5 and the hr mutants used in this study have been described previously (Harrison et al., 1977; Graham et al., 1978). Mutant hr3 belongs to complementation group I, and hr6 belongs to complementation group II. Titres of wt Ad5 and the hr mutants were determined by plaque assay on monolayers of 293 cells and the particle/p.f.u. ratio of these viruses was in the range of 50 to 100. Ratios of titres on 293 versus HeLa cells were typically in the range of 10^2 to 10^3 for both group I and II mutants.

Infection and labelling of cells. Confluent monolayers of KB cells in 150 mm culture dishes were infected with 5 p.f.u. of Ad5 wt or hr mutant per cell in 2 ml phosphate-buffered saline (PBS). After 30 min of adsorption at 37 °C, the cultures received 20 ml of α-MEM supplemented with 5% HS. At the appropriate time post-infection, the cells were rinsed once with PBS, and labelled with 50 μCi[^35]S]methionine in 4 ml of methionine-free medium. After the labelling period the cells were washed once with PBS and harvested by scraping.

Spinner cultures of exponentially growing KB cells were concentrated 10-fold and infected with the appropriate dilution of virus. After 30 min of adsorption at 37 °C the volume was adjusted to normal with spent culture medium which had been kept at 37 °C separately. For labelling, aliquots of 3 × 10^7 cells were removed from the culture flask and resuspended in 0.1 vol. methionine-free medium containing 75 μCi[^35]S]methionine. After labelling, the cells were washed once with PBS and harvested by centrifugation.

Antiserum. Sera were collected from hamsters bearing tumours induced by various Ad5-transformed cell lines. The cell lines included 14B (Williams, 1973), 983-2, an Ad5 Xhol-C fragment (0 to 16%) transformed hamster cell line, and 945-C1 and 954-C4, cell lines transformed by HindIII digests of Ad5 DNA (F. L. Graham, unpublished results). The tumour sera were prepared against these cell lines were either used individually or were pooled to provide a polyvalent antiserum which detected the presence of several major antigens in a given extract.

The techniques for production and characterization of mouse hybridomas secreting monoclonal antibodies to E2A antigens have been previously described (Killington et al., 1981). Briefly, 5 × 10^6 disrupted cells harvested 8 h after infection with Ad5 were injected in complete Freund's adjuvant intraperitoneally (i.p.) and intramuscularly into BALB/c mice. Two subsequent i.p. boosts of virus-infected cells without adjuvant at 2-week intervals were given and spleen cells were fused 3 days after the second boost. Positive polyclones were detected by ELISA, cloned, and injected i.p. into Pristane-primed BALB/c mice. The resulting ascitic fluid containing monoclonal antibodies was used in this study.

Immunoprecipitation. Immunoprecipitations were carried out by a modification of the procedure described by Schaffhausen et al. (1978) using Protein A–Sepharose beads. Briefly, the labelled cells were washed once with PBS and lysed in 1 ml of precipitation buffer (20 mm-Tris–HCl pH 7.5, 10% Triton X-100, 1% sodium deoxycholate, 150 mm-NaCl, 0.2% SDS) per 10^7 cells. After 20 min at 4 °C, the cell lysate was sonicated (Biosonic III, setting 30) and centrifuged at 10000 g for 10 min. Usually, 10 μl of antiserum and 30 μl of equilibrated and settled Protein A–Sepharose beads were added per ml of the supernatant. After 3 h of constant mixing at 4 °C, the beads were recovered by low-speed centrifugation, washed three times with equal volumes of a wash buffer (50 mm-Tris–HCl pH 7.5, 250 mm-LiCl, 0.1% 2-mercaptoethanol) and suspended in 50 μl of electrophoresis sample buffer.

SDS-polyacrylamide gel electrophoresis. The immunoprecipitated proteins were solubilized in sample buffer (0-625 mm-Tris–HCl pH 6-8, 2% SDS, 0.1% 2-mercaptoethanol, 10% glycerol, 0.2% bromophenol blue) and analysed by discontinuous gel electrophoresis (SDS–PAGE) (Laemmli, 1970) on 1-5 mm-thick, 180 mm-long gels consisting of 12-5% or 15% acrylamide and a 5% stacking gel. Thirty μl of samples were electrophoresed at 80 V for 12 h and radioactive bands were detected by autoradiography of dried gels. Relative incorporations of radioisotope into protein bands were measured by scanning autoradiograms with a Joyce-Loebl double-beam recording densitometer.

RESULTS

Immunoprecipitation of early viral proteins with anti-tumour serum

Analysis of viral proteins synthesized during the early phase of adenovirus lytic infection requires techniques which will allow detection of viral products within the considerable
background of cellular protein synthesis. Labelling *in vivo* with $[^{35}$S]methionine followed by immunoprecipitation with anti-tumour sera has been used by a number of groups to isolate viral gene products from infected and transformed cells (Johansson *et al.*, 1978; Lassam *et al.*, 1978; Green *et al.*, 1979; Schrier *et al.*, 1979; Ross *et al.*, 1980a). This procedure requires that appropriate sera be available in order to precipitate and analyse an array of viral proteins. Fig. 1 shows that different anti-tumour sera used to immunoprecipitate viral antigens labelled from 7 to 9 h post-infection varied greatly in avidity for any particular protein. Sera derived from hamsters bearing tumours seemed to have specificities which in general reflected the expression of viral proteins in the transformed cells used to induce the tumour (Rowe *et al.*, 1984). Fig. 1 shows that tumour serum made in response to 954-C4 cells was directed principally against the 19K antigen. Different batches of 954-C4 anti-tumour serum (as well as other anti-tumour sera and normal hamster sera) often showed varying degrees of reactivity with additional proteins. These were also precipitated from mock-infected cell extracts and were therefore not virus-specific. The immune response to 983-2 cells was against the E1B 58K antigen, and a complex of several proteins migrating as a diffuse band around 44K. The 945-C1 serum was almost monospecific for the 14K protein, although a low avidity for the 44K and 19K antigens was also observed, and finally, 14B tumour serum was primarily active against the 58K antigen but also appeared to react weakly with a number of other antigens. None of these bands which were immunoprecipitated with various anti-tumour sera was detected by immunoprecipitation with normal hamster serum (Fig. 1).

The characterization of a variety of transformed cell lines for viral DNA and protein content and analysis of tumour antigen synthesis in cells infected with various deletion mutants has permitted the tentative identification of the regions of the viral genome that encode the various viral proteins seen in Fig. 1 (Downey *et al.*, 1983; Rowe *et al.*, 1983a). In those studies the 44K complex was assigned to E1A, the 58K and 19K products to E1B, and the 14K protein to E4, assignments which were consistent with the reports of other workers (Ross *et al.*, 1980a; Bos *et al.*, 1981; Smart *et al.*, 1981; Sarnow *et al.*, 1982). By pooling the sera whose specificities are shown in Fig. 1, a polyvalent serum was made which was capable of immunoprecipitating the E1A 44K complex, the E1B 58K and 19K products and the E4 14K protein. With this pooled serum it was possible to examine the time course of expression of these early transcription units at the level of translation in cells infected with wt Ad5 or with hr mutants.

**Kinetics of early protein synthesis during wt infection**

In a preliminary time course experiment, KB cells grown in suspension and infected with wt virus at 50 p.f.u./cell were pulse-labelled for 1 h with $[^{35}$S]methionine every hour until 12 h after infection. The synthesis of late polypeptides was detected in whole cell extracts beginning at 8 h (data not shown). Prior to 8 h the pattern of protein synthesis in infected cells as determined by polyacrylamide gel analysis of 1 h pulses with $[^{35}$S]methionine was indistinguishable from that of uninfected controls with the exception of the appearance of detectable levels of the 72K protein (see below). Aliquots of the infected cell extracts were immunoprecipitated with the polyvalent tumour antiserum described above and the antigens recovered were separated on a polyacrylamide gel (Fig. 2). It was possible to detect synthesis of E1A products within 3 h of addition of the virus to the cells and these proteins migrated as a diffuse band in the region of 44K as has been previously observed (Harter & Lewis, 1978; Ross *et al.*, 1980a; Persson *et al.*, 1981). Maximal rates of synthesis occurred by 5 h post-infection and then declined until at 10 h post-infection the band was no longer detectable. Since E1A is the first region to be transcribed (Nevins *et al.*, 1979) the rapid appearance of E1A translation products was expected, but the decrease in protein synthesis after 5 h contrasts with reports of continuous transcription and accumulation of cytoplasmic message throughout infection (Nevins *et al.*, 1979; Shaw & Ziff, 1980; Spector *et al.*, 1980; Persson *et al.*, 1981). Maximal rates of synthesis occurred by 5 h post-infection and then declined until at 10 h post-infection the band was no longer detectable. Since E1A is the first region to be transcribed (Nevins *et al.*, 1979) the rapid appearance of E1A translation products was expected, but the decrease in protein synthesis after 5 h contrasts with reports of continuous transcription and accumulation of cytoplasmic message throughout infection (Nevins *et al.*, 1979; Shaw & Ziff, 1980; Spector *et al.*, 1978).

The kinetics of expression of the early region 1B 19K protein were very similar to those of the E1B 58K protein. These products were first detected at approximately 6 h and continued to be made in readily detectable quantities up to 12 h. For both proteins, the rate of synthesis was maximal at approximately 8 h post-infection and then expression declined to a lower level which...
Fig. 1. Autoradiogram of polyacrylamide gel-separated antigens immunoprecipitated from Ad5-infected cells. Extracts prepared from infected cells labelled with [35S]methionine from 7 to 9 h post-infection were immunoprecipitated with various anti-tumour sera as described in Methods. V, Purified virus marker; baby hamster kidney cell lines used to induce tumours were: 14B, transformed by a ts Ad5 mutant (Williams, 1973); 983-2, transformed by XhoI-C fragment (0 to 16%); 954-C4 and 945-C1, transformed by HindIII digest of Ad5 DNA; NI, non-immune serum. Roman numerals refer to various structural components, and numbers refer to mol. wt. x 10^-3 as determined by SDS–PAGE.

was maintained well into the late phase. These observations are consistent with maximal rates of E1B RNA synthesis having been reported to occur around 7 h post-infection (Nevins et al., 1979) although the rate of accumulation of E1B 13S mRNA increases 50-fold relative to the accumulation of E1B 22S message at late times. Infected cells labelled with [35S]methionine for 1 h at 24 h post-infection show synthesis of 19K but not 58K (data not shown) even though the 22S message has been shown to persist in minute quantities at late times (Spector et al., 1978).
The E4 14K protein first appeared at 4 h post-infection and reached a maximal rate of synthesis between 6 and 7 h followed by a sharp decline. This pattern is virtually identical to that reported by Neuwald et al. (1977) and both our results and those of Neuwald et al. (1977) conform to the pattern of transcription that has been reported for region E4 (Nevins et al., 1979). Thus, the rates of translation of E1B and E4 proteins observed in this study are in accordance with the available data on the rates of RNA transcription and accumulation and protein synthesis from these regions. However, the E1A translation pattern, which showed a cut-off after about 7 h, was in contrast with the E1A transcription and mRNA accumulation reported to occur at much later times (Nevins et al., 1979; Shaw & Ziff, 1980; Spector et al., 1978).

Effect of hr mutations on early protein synthesis

Investigators of viral RNA synthesis utilizing deletion and hr mutants and translational inhibitors have identified a role for E1A products in the stimulation of early Ad5 transcription (Berk et al., 1979; Nevins, 1981; Shaw & Ziff, 1982). To examine this control of expression as manifested at the translational level, the rate of translation of tumour antigens from regions E1A, E1B and E4 was analysed and compared for KB cells infected with wt virus or the E1A mutant hr3 (Fig. 3). Expression of E1A proteins in hr3-infected cells was characterized by a longer lag period relative to wt kinetics and two separate peaks of E1A protein synthesis appeared at 7 and 10 h post-infection before a cessation of translational activity nearly 12 h post-infection (Fig. 3a). The normal early peak of expression of E1B 19K and E4 14K was absent from hr3 infections, but at late times translation of both proteins appeared to have recovered to levels comparable to wt-infected cells (Fig. 3b, c). Synthesis of the E1B 58K antigen was not detected in immunoprecipitates of hr3-infected cells during the course of this experiment. It was occasionally possible to detect synthesis of reduced amounts of 58K in hr3 infections particularly at higher m.o.i. where the leakiness of this hr mutant was also characterized by the ability to synthesize increased amounts of viral DNA (Rowe & Graham, 1981). The experiment illustrated in Fig. 3 has been carried out a total of four times. The data presented are representative and although points along the curve varied somewhat from experiment to experiment, the general trends in the kinetics and especially the differences between mutant and wt infections were reproducible. The effects of the hr3 lesion on early viral protein synthesis are consistent with the proposed role of E1A gene products in the control of early viral gene expression.
Fig. 3. Kinetics of protein synthesis in hr mutant-infected KB cells. Infected cells were pulse-labelled at the times (h) shown with [35S]methionine as described in the legend to Fig. 2. Portion of the autoradiogram containing the viral antigen analysed is shown above the plot of the densitometer scans: Δ, wt; ●, hr3; ▲, hr6. (a) 44K; (b) 19K; (c) 14K.
To determine whether a product of early region 1B was involved in the regulation of early gene expression, a similar comparison of translational activity was performed using the E1B mutant hr6. This mutant, although it shows a multiplicity-dependent leakiness for virus production, synthesizes no immunoprecipitable 58K antigen even at high m.o.i. (Lassam et al., 1979; Ross et al., 1980b). Synthesis of the E1A 44K protein in hr6-infected cells peaked at 5 h after infection, similar to wt, but peaked at a lower maximal rate. After decreasing to a minimum level of expression at 8 h post-infection, 44K protein synthesis increased again over the next 4 h and
showed no indication of abatement by 12 h post-infection in contrast to synthesis in wt-infected cells which had shut off E1A translation by 10 h post-infection (Fig. 3a). The translation of the E1B 19K increased slowly over the 8 h period from 4 to 12 h post-infection but did not show the early peak of expression characteristic of wt infection. The pattern of E4 14K synthesis was also perturbed by the hr6 defect which caused a lower maximal rate of expression and shifted the peak to a later time after infection (Fig. 3c). While it is difficult at present to suggest a single mechanism by which all these regulatory effects occur, these results suggest that E1B may encode some regulatory function which modulates expression of early viral proteins at early times after infection.
Kinetics of E2A 72K synthesis in wt and hr mutant infections

The 72K DBP encoded by the E2A region is an abundant early phosphoprotein (van der Vliet & Levine, 1973; Levinson et al., 1977; Axelrod, 1978) with a complex role in adenovirus gene expression (Klessig & Grodzicker, 1979; Nevins & Winkler, 1980; Babich & Nevins, 1981; Nicolas et al., 1982) and DNA replication (van der Vliet et al., 1975; Nagata et al., 1982). Although the kinetics of synthesis of this protein may be measured directly by polyacrylamide gel electrophoresis of pulse-labelled infected cell extracts (Neuwald et al., 1977; Axelrod, 1978), most workers enhance detection of E2A products by immunoprecipitation with polyvalent or monoclonal IgG antibody, H2-19 (see Methods) which specifically immunoprecipitates the major forms of the DBP (Axelrod, 1978; Jeng et al., 1977) as well as some of the minor related species (Fig. 4; Asselbergs et al., 1983). For this study we have used a murine monoclonal IgG antibody, H2-19 (see Methods) which specifically immunoprecipitates the major forms of the DBP (Axelrod, 1978; Jeng et al., 1977) as well as some of the minor related species (Fig. 4; Asselbergs et al., 1983). Since the subset of E2A polypeptide species immunoprecipitated by H2-19 appear to be the same products detected by the rabbit antiserum prepared against purified DBP, it is possible that the site recognized by the monoclonal antibody is within the sequences corresponding to amino acids 170 to 240 which bear the antigenic determinants recognized by rabbit antiserum (Asselbergs et al., 1983). The monoclonal antibody, which was obtained in high titer from murine ascites tumors, was used to immunoprecipitate E2A proteins from extracts of cells infected with wt, hr3 or hr6 and then pulse-labelled for 1 h at various times after infection (Fig. 5). Synthesis of E2A proteins (72K, 67K, 50K and 48K) in wt-infected cells was detected 3 h post-infection, was maximal by 7 h and subsequently declined. The kinetics of E2A translation in cells infected with hr3 (E1A mutant) showed no early peak of expression but the rate of synthesis increased slowly to wt levels by 12 h post-infection, a pattern very similar to the kinetics of synthesis of E1B 19K and E4 14K in hr3-infected cells (Fig. 3b, c). Synthesis of E2A proteins in hr6-infected cells was not severely affected and followed a pattern similar to that seen for E4 14K expression. Previously, it had been suggested that the E2A DBP was overproduced in hr7 (an E1B mutant of the same complementation group as hr6) infected cells assayed by a 4 h pulse label from 12 to 16 h after infection (Ross et al., 1980b). Our results (Fig. 5) indicate that there was little difference in the levels of expression of 72K in hr6- and wt-infected cells except that the peak of synthesis in hr6-infected cells may be slightly shifted to late times. This could result in persisting 72K synthesis in hr6 infections at times when E2A expression has been curtailed in wt-infected cells.

DISCUSSION

We have studied the kinetics of early gene expression during adenovirus infection at the level of translation. The time of appearance of proteins from early region 1A, 1B, 2 and 4 was distinct in each case, as was the occurrence and duration of the maximal rate of expression (Fig. 2). The reduction of E1A protein synthesis during the course of wt infection is qualitatively similar to the reduction which was seen in protein synthesis from other early regions, but temporally distinct, reaching very low levels at the same time as peak expression of E1B and E2. This effect on E1A gene expression is likely to be mediated at the level of translation, as E1A 13S and 12S mRNAs have been reported to accumulate in the cytoplasm at a constant rate throughout infection (Spector et al., 1978; Wilson & Darnell, 1981).

The kinetic studies with the E1A mutant hr3 revealed a delay in protein synthesis and decreased levels of translation of E1B, E2 and E4. While these results are in general agreement with the proposed role of E1A functions for the regulation of expression of other early regions at the level of transcription (Berk et al., 1979; Ricciardi et al., 1981; Nevins, 1981; Jones & Shenk, 1979a), E1A protein synthesis in hr3-infected cells peaked twice within 12 h after infection; this contrasted with a single peak for wt-infected cells (Fig. 3a) and may indicate a role for E1A functions in the control of E1A expression. It is not clear from this study whether E1A autoregulation acts at the transcriptional or post-transcriptional level or whether this effect is indirectly caused by reduced amounts of other early gene products.

During lytic infection, the E1B hr6 defect was characterized by deviations from normal early gene expression for all products examined. In general, the mutant appeared to induce delayed...
and reduced maximal levels of protein synthesis from E2 and E4 and no early phase of E1B 19K expression (Fig. 3 and 5). As with the hr3 mutant, the kinetics of E1A protein synthesis in hr6-infected cells were more complex than in wt-infected cells, indicating a failure of the normal regulation of E1A expression but leaving open the question of how this effect is mediated. Mutant hr6 infections of HeLa cells are also characterized by reduced levels of late gene expression (Lassam et al., 1978), yields of infectious virus which are reduced by as little as one order of magnitude (Rowe & Graham, 1981) and marked multiplicity-dependent leakiness. All of these effects appear to be due to the failure of hr6 to synthesize a 58K E1B protein, suggesting that this product may be required not for execution of the viral genetic programme but for the efficient expression of viral genes at both early and late times after infection. Cell fractionation and immunofluorescence studies of the 58K have suggested that this protein is associated with the nucleus at late times (Rowe et al., 1983b). This high level of late nuclear association parallels the reported restriction of host mRNA transcripts to the nucleus late in infection (Beltz & Flint, 1979). A role for the 58K protein in this process could explain most of the phenotypic effects of hr6 and other E1B mutants.

The synthesis of greatly reduced amounts of the E1B 19K protein early after hr6 infection (Fig. 3b) suggests that an autoregulatory function may reside within E1B. It thus remains unclear whether the phenotypic effects described for the hr6 mutant are directly attributable to a lack of 58K expression or indirectly the result of reduced levels of E1B 19K expression. More precise mapping of the hr6 lesion in the 58K coding sequences and the characterization of mutants in other parts of E1B will be important to an understanding of this potential autoregulatory effect. The finding of normal levels of early cytoplasmic mRNA in hr7-infected cells (Berk et al., 1979) indicates that this control may be at a post-transcriptional level.

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Kinetics of synthesis of early Ad5 proteins


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