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Mapping of functional domains in adenovirus E1A proteins

(*Escherichia coli* expression system/microinjection/nuclear transport/gene activation/c-*myc*)

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ABSTRACT **We have modified the *E1A* gene of human subgroup C adenovirus by introducing deletions in its coding sequence. Various truncated E1A proteins were expressed in *Escherichia coli*, purified, and microinjected via glass capillaries into Vero cells. We monitored their movement from the cell cytoplasm to the nucleus and their ability to induce expression of H5*dl*312, an adenovirus *E1A* deletion mutant. Our results show that the carboxyl terminus of E1A contains sequences essential for rapid and efficient nuclear localization. Essential information for efficient H5*dl*312 complementation is contained in an internal region, comprising sequences of both exons of the *E1A* gene. A first exon-encoded region, however, is sufficient to induce low levels of adenovirus gene expression. Information for nuclear localization and for H5*dl*312 complementation are therefore encoded by distinct domains of the *E1A* gene. In addition, we determined that the human c-myc product was unable to complement H5*dl*312.**

The products of *E1A* gene initiate the infectious cycle of adenovirus by activating the other early transcription units. They also modulate the transcription of other viral and of certain cellular genes, and they play an important role in malignant cell transformation (1). The human adenovirus subgroup C *E1A* gene is transcribed into two mRNAs, 12S and 13S, which share common 5′ and 3′ termini but differ internally by the size of the intron removed. The 12S mRNA encodes a protein of 243 amino acids, which differs from the 289 amino acid 13S mRNA product by an internal deletion of 46 amino acids (2).

The pleiotropic nature of *E1A* activity has stimulated interest in mapping functional domains within the gene. The most commonly used approach has been to study the effect of mutations within the gene that alter expression in specific ways (1). We have opted instead to work directly with the E1A proteins and to study the effects of various structural alterations on their functions. Applying the proteins directly to the cell, rather than the modified DNAs that encode them, has the advantage that controls are bypassed that may influence expression of altered genes. In addition, microinjection allows us to place precise amounts of these proteins in the cell compartment of choice—that is, in the nucleus or in the cytoplasm, a prerequisite for determining the effects of various mutations on the intracellular location and the function of a given E1A protein.

Previously, we have constructed plasmid expression vectors that permit regulated and high-level expression in *Escherichia coli* of the products of the human subgroup C adenovirus E1A 13S and 12S mRNAs. Using microinjection to introduce these purified proteins into mammalian cells, we find that the 12S mRNA product, like the 13S product, localizes rapidly to the cell nucleus and stimulates early and late adenovirus gene expression (3–6).

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This report focuses on the functional domains within the E1A protein that are responsible for nuclear localization and for complementation of the viral *E1A* deletion mutant. We deleted various portions of the *E1A* coding sequence, expressed the altered sequences in *E. coli*, and obtained a series of truncated E1A proteins. Each of the proteins was purified and examined in our microinjection assays. We find that rapid nuclear localization of both the 12S and 13S mRNA products requires the presence of an intact carboxyl terminus. In contrast, an internal region of the E1A proteins is required for H5*dl*312 complementation. We also show that the human c-myc gene product, a protein that has been structurally and functionally related to E1A (7–10), is unable to complement H5*dl*312.

MATERIALS AND METHODS

Plasmid Vector Construction. The plasmid expression vector pAS1 (11) was used for the expression in *E. coli* of E1A-derived proteins. A unique *Bam*HI site in pAS1 allows DNA segments of the *E1A* coding sequence (Fig. 1) to be fused precisely to the translation initiation codon on the vector. The construction of pAS1 (12) derivatives that encode E1A insertion and deletion mutant proteins is detailed below. The products of these expression vectors are represented schematically in Fig. 1. The correct constructions were identified by fine restriction mapping and by expression of the E1A product as detected by immunoblot analysis using antiserum specific to full-length E1A 13S mRNA product (4).

pAS1-E1A420. Plasmid pAS1 (11) was cut with *Bam*HI, blunt-ended with DNA polymerase (Klenow), treated with calf alkaline phosphatase, and ligated with a 1748-bp *Pvu* II fragment from pJN20 (3), which contains sequences encoding amino acids 22–289 of E1A, to yield pAS1-E1A220. In pAS1-E1A220, *Bam*HI sites are recreated at both ends of the insert and the insert is oriented such that the 5′ end of the *E1A* coding sequence is adjacent to, but not in-frame with, the *c*II translation initiation codon on the vector. The 1542-bp *Hpa* I–*Nru* I DNA fragment 3′ to the *E1A* coding sequence was deleted by restriction of pAS1-E1A220 with *Hpa* I and *Nru* I and ligation to yield pAS1-E1A320. The *E1A* coding sequence was positioned in-frame with the *c*II translation initiation codon on the expression vector by *Bam*HI restriction of pAS1-E1A320, followed by treatment with phosphatase, limited digestion with mung bean exonuclease, and ligation. pAS1-E1A420 encodes a product that differs from the authentic E1A 13S mRNA product by deletion of 21 amino acid residues (2–22) at the E1A amino terminus.

pAS1-E1A610. pAS1-E1A610 was constructed by cutting pAS1-E1A410 (3) at the unique *Xba* I site, filling in the 5′ overhanging ends with DNA polymerase, and ligation. This procedure creates an in-frame termination codon within the *E1A* coding sequence and results in deletion of 67 amino acid

Abbreviation: bp, base pair(s).

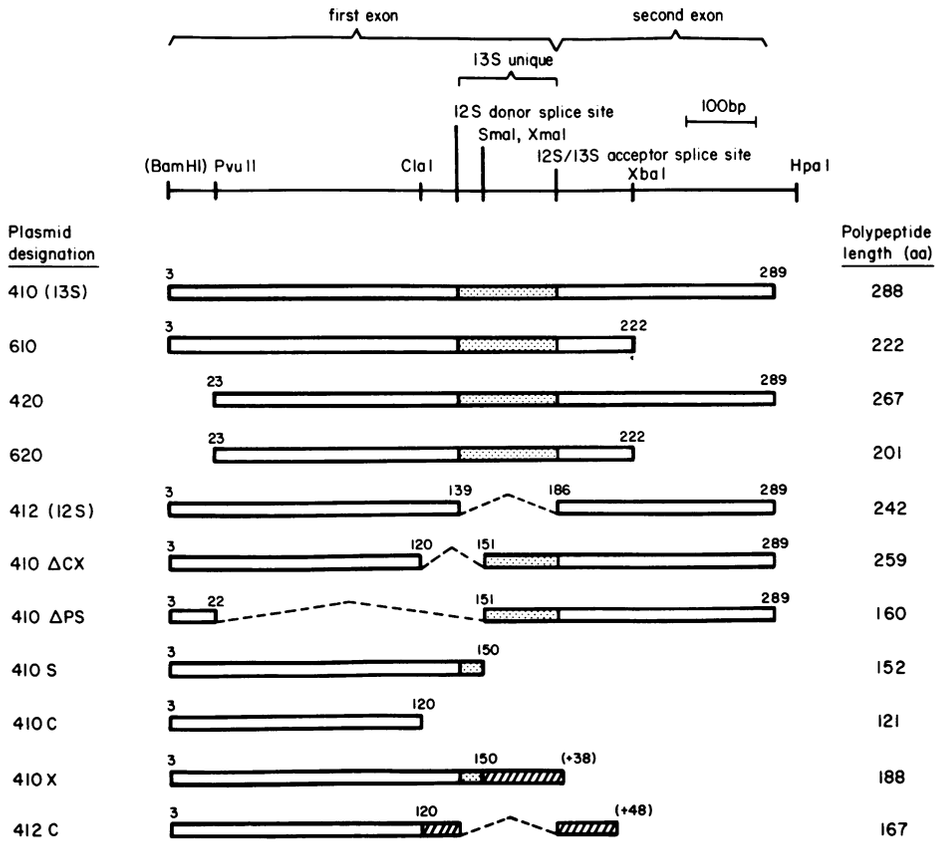

FIG. 1. Schematic representation of insertion and deletion mutant E1A gene products expressed in *E. coli*. Plasmid expression vectors encoding insertion and deletion mutant E1A proteins were constructed as described. The numbers of the first and last amino acid encoded by segments of *E1A* coding sequence (open thick line) that were fused directly to the λ *c*II translation initiation codon supplied by the expression vector (3) are indicated. Amino acids unique to the 13S mRNA product (stippled) as well as missense (hatched) and deleted E1A-specific (dashed line) amino acids are indicated. Also shown are the regions of E1A protein encoded by the first exon and second exon, the region unique to the 13S mRNA product, restriction endonuclease sites used in the construction of the plasmid expression vectors, and the amino acid length of the E1A-derived polypeptides expressed in *E. coli*. aa, Amino acids; bp, base pairs.

residues (223–289) and insertion of a single serine residue at the E1A carboxyl terminus.

pAS1-E1A620. pAS1-E1A620 was generated by cutting pAS1-E1A420 with *Xba* I, filling in the 5′ overhanging ends with DNA polymerase, and ligation. This plasmid encodes a product that differs from the full-length E1A 13S mRNA product by the deletion of 21 amino acid residues at the amino terminus (2–22) and deletion of 67 amino acid residues (223–289) and insertion of a serine residue at the E1A carboxyl terminus.

pAS1-E1A410ΔCX. pAS1-E1A410ΔCX was constructed by using pAS1-E1A410 by deletion of the DNA segment between the *Cla* I and *Xma* I sites within the *E1A* coding sequence. pAS1-E1A410 contains two *Cla* I sites, one within the *E1A* coding sequence and one within the pBR322 region of the vector. The *Cla* I site in the pBR322 region of pAS1-E1A410 was first deleted by restriction with *Eco*RI and *Hin*dIII, filling in the 5′ overhanging ends with DNA polymerase, and ligation. The *Eco*RI- to *Hin*dIII-deleted pAS1-E1A410 was cleaved with *Cla* I and *Xma* I and the 5′ overhanging ends were filled in with DNA polymerase and ligated to yield pAS1-E1A410ΔCX. This plasmid encodes an E1A product with an internal deletion of 30 amino acid residues (121–150) replaced by an alanine residue.

pAS1-E1A410ΔPS. pAS1-E1A410ΔPS was generated by deletion of the DNA segment between the *Pvu* II and *Sma* I restriction endonuclease sites within the *E1A* coding sequence of pAS1-E1A410. pAS1-E1A410 contains one *Sma* I site and two *Pvu* II sites, one within the *E1A* coding sequence and one within the pBR322 region of the vector. pAS1-E1A410 was cleaved with *Sma* I and *Pvu* II and the largest two fragments were isolated and ligated to yield pAS1-E1A410ΔPS. This plasmid encodes an E1A-derived product with an internal deletion of 128 amino acid residues (23–150).

pAS1-E1A410S. pAS1-E1A410S was constructed by inserting a 12-bp synthetic oligonucleotide, which contains a unique *Hpa* I site and translation termination codons in the three possible reading frames, at the *Sma* I site within the *E1A* coding sequence. The 5′ to 3′ sequence of the synthetic oligonucleotide is CTAGTTAACTAG. pAS1-E1A410 was cut with *Sma* I, treated with phosphatase, and ligated in the presence of the synthetic oligonucleotide to yield pAS1-E1A410S. This plasmid encodes a product with 139 amino acid residues (151–289) deleted from the carboxyl terminus of the E1A 13S mRNA product and replaced with three missense residues (Leu-Val-Asn).

pAS1-E1A410C. pAS1-E1A410C was generated by inserting the 12-bp synthetic oligonucleotide containing translation termination codons at the *Cla* I site within the *E1A* coding sequence. pAS1-E1A410 contains two *Cla* I sites and the *Cla* I site within the pBR322 region of the vector was deleted. The *Eco*RI- to *Hin*dIII-deleted pAS1-E1A410 was cut with *Cla* I, filled in with DNA polymerase treated with phosphatase, and ligated in the presence of the synthetic oligonucleotide to yield pAS1-E1A410C. This plasmid encodes a protein with 169 amino acids deleted from the carboxyl terminus (121–289) and replaced with two missense residues (Ala-Ser).

pAS1-E1A410X. pAS1-E1A410X was constructed by cleaving pAS1-E1A410 at the unique *Xma* I site within the *E1A* coding sequence, filling in the 5′ overhanging ends with DNA polymerase, and ligation. This results in the creation of an *Xma* III site and a shift in reading frame at the *Xma* I site.

pAS1-E1A410X encodes a product with 138 amino acids deleted from the E1A carboxyl terminus (152–289) and replaced with 38 missense residues.

pAS1-E1A412C. pAS1-E1A412C was generated by cutting pAS1-E1A412 (6) at the unique *Cla* I site, filling in the 5′ overhanging ends with DNA polymerase, and ligation. This procedure creates an *Nru* I restriction site and causes the *E1A* coding sequence to be frame-shifted at the *Cla* I site. In the product of pAS1-E1A412C, 169 amino acids (121–289) are deleted from the carboxyl terminus and are replaced by 48 missense amino acid residues.

Other Procedures. The expression in *E. coli* of E1A insertion and deletion mutant proteins encoded by derivatives of pAS1 (Fig. 1) was controlled transcriptionally by nalidixic acid induction by using the phage λ P_L promoter on the vector and the defective lysogenic host AR120, which supplies *c*I (13). The E1A-derived protein is partially purified and represents about 60% of the total protein in the preparation. E1A protein concentration and purity were determined by Coomassie blue staining of $NaDodSO_4$/polyacrylamide gels. Microinjection of proteins in Vero cells, immunofluorescence, immunoblotting, and immunoprecipitation procedures have been described (4).

RESULTS

Expression in *E. coli* and Isolation of Mutant E1A Proteins. We have constructed a series of plasmid vectors that encode and express in *E. coli* insertion and deletion mutant products of the human adenovirus subgroup C *E1A* gene. Several plasmid derivatives of pAS1-E1A410 were constructed that encode variant E1A proteins differing from the E1A 13S mRNA product by the deletion of amino-terminal, carboxyl-terminal, or internal amino acid residues. Deletions were introduced into the *E1A* coding sequence by using several unique restriction endonuclease sites within the *E1A* coding sequence. The variant E1A protein products of these expression vectors are shown in Fig. 1.

The expression of each of the E1A-derived protein products listed in Fig. 1 was induced in *E. coli* and these proteins were isolated by using a rapid procedure developed previously for the isolation of E1A 13S protein (4). Each of the partially purified E1A-derived proteins is obtained as a single major immunoreactive polypeptide species when analyzed by immunoblotting (4) (data not shown).

Intracellular Localization of Truncated E1A Proteins. We have analyzed the effect of structural alterations of the E1A 13S mRNA product on its antigenic stability and on its ability to localize to the cell nucleus after microinjection into somatic cells. Previously, we showed that both the E1A 13S and 12S mRNA products localize quantitatively to the nucleus of monkey kidney cells (Vero) within 15–30 min after microinjection of the protein into the cell cytoplasm (4–6). Each of the purified E1A proteins (Fig. 1) was injected into the cytoplasms of Vero cells. Cells were analyzed at various times after injection by immunofluorescent staining by using a polyclonal antibody directed against the E1A 13S mRNA product (4). This E1A-specific antiserum detects each of the mutant E1A proteins.

The results are shown in Table 1. Proteins E1A 420, 410ΔPS, and 410ΔCX, like the E1A 12S and 13S proteins, localized rapidly (within 30 min) to the nucleus after cytoplasmic microinjection. These variant E1A proteins contain amino-terminal or internal deletions but have unaltered carboxyl terminus. In contrast, each of the E1A variants with carboxyl-terminal deletions, including E1A 610, 620, 410X, 412C, 410S, and 410C, localized to the nucleus very slowly. For example, E1A 610 and 620, which have 67 carboxyl-terminal E1A amino acid residues deleted, took 7–8 hr to fully localize to the nucleus after cytoplasmic microinjection.

Table 1. Intracellular location of microinjected E1A proteins as a function of time after cytoplasmic microinjection

	Location at time (hr) after injection									
Protein	¼	½	1	2	3	4	5	6	7	8
410	NC	N	N	N	N	N	N	N	N	N
412	NC	N	N	N	N	N	N	N	N	N
420	NC		N	N	N	N	N	N	N	N
410ΔCX	NC	NC	N			N	N		N	N
410ΔPS	NC	NC	N	N	N	N	N	N		
610		NC		NC	NC		NC	NC		N
620		C		C		NC	NC	NC	N	N
410X		NC	NC		NC		NC		NC	
412C		NC					NC		NC	
410S	C	C	C		C	C	NC		NC	NC
410C	C	C	C	C	C	C	NC	NC	NC	NC

E1A-derived proteins were injected into the cytoplasm of Vero cells. At given times thereafter, cells were analyzed by E1A-specific immunofluorescent staining. Positive staining is symbolized as N (nuclear), C (cytoplasmic), or NC (both nuclear and cytoplasmic).

Function of Mutant E1A Proteins in the H5*dl*312 Complementation Assay. We examined the ability of each of the E1A variant proteins to induce gene expression of the defective adenovirus H5*dl*312, in which the promoter region and a large part of the coding sequence of the *E1A* gene is deleted. Because it lacks *E1A*, H5*dl*312 produces very low levels of viral transcripts at early times after low-multiplicity infection (14, 15). As a consequence of this defect, H5*dl*312 also exhibits a low level of DNA replication and poor expression of the major late transcription unit. We have developed a sensitive and quantitative indirect assay for the ability of *E1A* to stimulate adenovirus H5*dl*312 gene transcription. In this assay, H5*dl*312-infected Vero cells are microinjected with E1A protein and expression of viral coat protein products of the major late transcription unit is quantitated by immunoprecipitation (4). We measure the ability of E1A protein to stimulate sufficient early gene transcription to allow replication of the virus and subsequent expression from the major late transcription unit. The assay is very sensitive because the coat protein signal is amplified as a consequence of viral replication and increased gene copy number in the cell. At the multiplicity of H5*dl*312 infection used, viral coat proteins are not detected in the absence of microinjected functional E1A protein. Previously, we demonstrated that the level of H5*dl*312 hexon protein expressed is proportional to the concentration of either E1A 13S or 12S protein microinjected over a range of 0.1–3 mg/ml (5, 6).

To quantitate and compare the functional activities of the various deletion mutant E1A proteins, H5*dl*312 hexon expression was measured in cells as a function of the concentration (0.2–2 mg/ml) of the microinjected E1A-derived protein (Figs. 2 and 3). From immunoblot analysis we estimated the amount of E1A protein in injected and infected cells. We find that there are 10^5–10^6 monomers of 12S and 13S protein in wild-type infected HeLa cells (50 plaque-forming units per cell), which is comparable to the amounts of E1A protein that we are introducing into the cell by microinjection. The level of H5*dl*312 hexon expressed was normalized for the number of viable microinjected cells by reference to certain cellular proteins that were unspecifically precipitated by the coat protein antibody (Fig. 2).

Surprisingly, the E1A mutant protein 610, which lacks 67 carboxyl-terminal residues, was able to induce H5*dl*312 expression nearly as efficiently as the E1A 13S protein (Fig. 3), even though this protein moves to the nucleus much more slowly than the E1A 13S or 12S proteins (Table 1). At the time we assay hexon expression (22 hr after microinjection) the

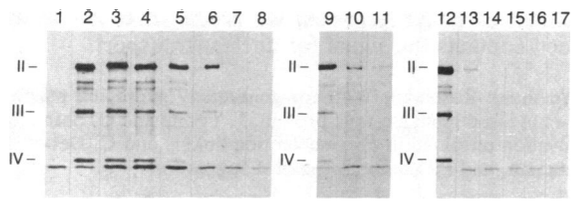

FIG. 2. Adenovirus coat protein expression in Vero cells infected with 200 plaque-forming units per cell of H5*dl* 312 and injected with E1A-derived proteins. Protein was injected at 2–4 hr after infection, cells were labeled for 2 hr with [^{35}S]methionine at 22 hr after infection, and the major viral coat proteins were immunoprecipitated and analyzed by NaDodSO$_4$/PAGE (4). Lane 1, control; lanes 2, 9, and 12, 410; lane 3, 610; lane 4, 620; lane 5, 412; lane 6, 420; lanes 7 and 15, 410C; lanes 8 and 16, 412C; lane 10, 410ΔCX; lane 11, 410X; lane 13, 410S; lane 14, 410ΔPS; lane 17, c-myc. The indicated E1A-derived proteins were injected at a concentration of 1 mg/ml (lanes 2–9) or 2 mg/ml (lanes 10–16). The control lane represents cells injected with an extract of *E. coli* carrying the plasmid pAS1 (3). Cells in lane 17 were injected with a solution containing 3 mg of purified *E. coli*-expressed human c-myc protein per ml. Each lane represents an immunoprecipitate from about 50 cells. The major viral coat proteins hexon (II), penton (III), and fiber (IV) are indicated.

truncated protein has had sufficient time to reach the nucleus and stimulate the transcription of viral genes.

Deletion of 21 amino-terminal residues from E1A (protein E1A 420) only slightly reduced its ability to induce H5*dl* 312 expression (Fig. 3). This defect was only observed when the protein was injected at low concentration (0.2 mg/ml). Full induction of H5*dl* 312 expression was achieved when this protein was injected at concentrations of 0.5–1 mg/ml. We interpret this to mean that deletion of the first 21 amino-terminal residues only partially impairs the function of E1A and that this defect is overcome by increasing the concentration of the defective protein. Similar results were obtained with E1A 620, which is missing both its amino-terminal 21 and carboxyl-terminal 67 residues. Again, although nuclear localization is significantly retarded, this variant also shows only a partial defect in gene activation that is fully compensated by injection of higher protein concentration.

In an effort to further define domains of the E1A protein that are essential for H5*dl* 312 complementation, we analyzed the ability of the other truncated E1A proteins (Fig. 1) to induce H5*dl* 312 hexon expression. The mutant E1A protein

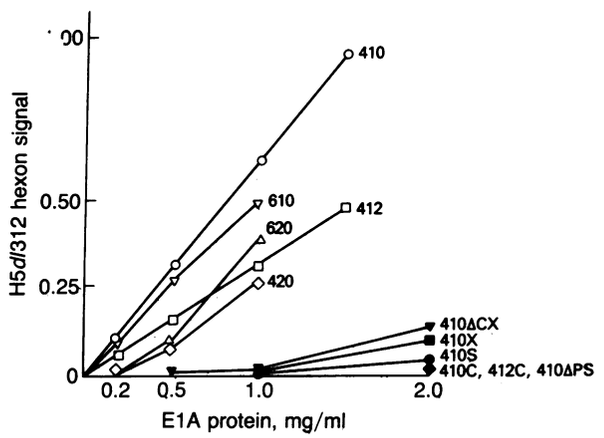

FIG. 3. H5*dl* 312 hexon protein expression as a function of the concentration of E1A-derived protein injected. Vero cells were infected with H5*dl* 312 and microinjected with E1A-derived protein, and viral coat protein expression was analyzed as described in the legend to Fig. 2. Hexon protein (band II) expression was quantitated by densitometry. The level of hexon, given in arbitrary units, is the mean value of three experiments.

that lacks 139 carboxyl-terminal residues replaced by 38 missense amino acids (E1A 410X) was unable to generate a hexon signal when injected at a concentration of 1.0 mg/ml or less. However, low levels of H5*dl* 312 hexon expression were clearly observed upon injection of 2.0 mg/ml. The 410S construct, which carries the same deletion replaced by only 3 missense residues, also gave a low H5*dl* 312 hexon signal. The mutant E1A proteins containing larger deletions of 169 carboxyl-terminal residues (E1A 410C and 412C) have completely lost H5*dl* 312-inducing activity, even when injected at concentrations up to 3 mg/ml.

In addition to the E1A mutants with amino-terminal and carboxyl-terminal deletions, we have examined the H5*dl* 312 hexon-inducing activity of two E1A mutants with internal deletions in the amino-terminal half of the molecule (E1A 410ΔCX and 410ΔPS). Both proteins were unable to induce H5*dl* 312 expression when injected at a concentration of 1 mg/ml or less. However, E1A 410ΔCX, the protein with the smaller deletion, induced low levels of H5*dl* 312 gene expression when injected at 2 mg/ml (Figs. 2 and 3).

Human c-myc Protein Does Not Complement Adenovirus H5*dl* 312. We have compared the ability of E1A and the human c-myc gene product to induce H5*dl* 312 gene expression. The E1A and myc gene products share certain structural (7) and functional (8–10, 16) similarities. We obtained a preparation of *E. coli*-expressed human c-myc protein (17) that has been shown to be active in a functional assay (18). When microinjected at concentrations up to 3 mg/ml, the c-myc protein was unable to induce H5*dl* 312 hexon synthesis (Fig. 2). The ability of the E1A 13S protein (injected at 0.2 mg/ml) or the protein E1A 410ΔPS (injected at 2 mg/ml) to induce H5*dl* 312 hexon expression was not affected by coinjection of c-myc protein (data not shown). Thus, the c-myc protein appears to be unable to either compete with the E1A 13S protein or help the deletion mutant E1A protein in the stimulation of adenovirus gene expression.

DISCUSSION

Our results clearly demonstrate that the E1A protein can be divided into separate functional domains. With respect to transcriptional activation as measured in the H5*dl* 312 complementation assay, the mutant E1A proteins fall into two categories. The first group, comprising proteins that have an unaltered core sequence within the confines of amino acid 23 at the amino-terminal and amino acid 222 at the carboxyl-terminal site, exhibit H5*dl* 312-inducing activity similar to the 12S and 13S E1A products. Our findings are in good agreement with studies on adenovirus mutants that had shown that E1A retains its regulatory function after deletion of 14 amino acids at the amino terminus or 87 amino acids at the carboxyl terminus (14, 19, 20). It should also be pointed out that three areas of conservation found among *E1A* sequences of several human and simian adenovirus serotypes are within the region left unaltered in these experiments (21). From the analysis of the proteins in this first group we conclude that a central region of the *E1A* gene, comprising sequences from both exons, is required for the effective regulatory function of E1A in the lytic cycle.

The second group of our mutant E1A proteins that lack part of this central region is inactive in the H5*dl* 312 complementation assay when tested at a concentration of 1 mg/ml. However, some of the proteins in this second group, such as E1A 410S, 410X, or 410ΔCX, exhibit low activity when tested at higher concentrations. Protein 410ΔCX carries a small deletion comprising amino acids 121–150. This protein does not efficiently induce H5*dl* 312 gene expression, implying that sequences within this region are crucial for the stimulating activity of the E1A protein. Since amino acids 121–139 are found in both the 13S and 12S proteins and since

the activity in our assay of the 12S protein is similar to that of the 13S protein, the critical segment might be even smaller—that is, from amino acids 121–139. A portion of the second exon of the E1A protein also contributes to the ability of the protein to complement H5*dl*312 virus. This is demonstrated by the fact that E1A 410S and 410X, which have unaltered first exon encoded regions, induce hexon protein synthesis poorly. As shown above, deletion of 67 amino acids from the carboxyl-terminus of the E1A protein does not impair its ability to induce hexon protein expression. In addition, previous studies have shown that an adenovirus mutant carrying a carboxyl-terminal deletion of 87 amino acids produces nearly wild-type levels of early viral mRNAs (14). Therefore, the essential information that the second exon of E1A supplies for H5*dl*312 complementation is most likely contained within a region that maps immediately downstream of the 12S/13S acceptor splice site. Our results indicate that the first exon of E1A encodes a domain that is sufficient to induce low levels of H5*dl*312 gene expression. Previous reports have also indicated that the first exon may encode a distinct functional domain (22–24). Additional mapping studies will be required to further define the regions of the E1A protein that are important for its gene activation function.

With respect to nuclear localization, our mutant E1A proteins again fall in two categories. All proteins in the first group have an intact carboxyl terminus, including E1A 420, 410ΔCX, and 410ΔPS. They behave like the 13S and 12S products in that they localize quantitatively to the nucleus within a short time after cytoplasmic injection. The second group, which comprises the remainder of the mutant proteins, lacks the carboxyl terminus. They also appear predominantly in the nucleus, albeit many hours after cytoplasmic microinjection. Apparently separate mechanisms guide these two groups of proteins to the nucleus. The fact that all of the E1A-derived proteins eventually localize to the nucleus suggests that they may retain affinity for nuclear components.

The rate of accumulation of a protein in the nucleus will depend on its size or diffusion rate, its rate of translocation across the nuclear membrane, and its affinity for a nuclear component (25). Our results show that the rapid nuclear accumulation of E1A is independent of the size or functional activity of the molecule. These results are consistent with an active mechanism for translocation of E1A into the nucleus that depends on recognition of a signal located in the carboxyl terminus of the protein. Analogous information has been detected in other eukaryotic nuclear proteins (25, 26). In simian virus 40 T antigen, there is a short internal region that functions as a signal for transport across the nuclear membrane (26). A similar internal region that includes the amino acid sequence Lys-Arg-Pro-Arg has been identified recently in polyoma large T antigen (W. D. Richardson, personal communication). This same sequence is also found at the carboxyl terminus of both adenovirus type 5 and 12 (27). As all mutant proteins that migrate rapidly to the nucleus contain an intact carboxyl terminus, we predict that this terminus indeed contains the signal for nuclear transport.

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