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A first exon-encoded domain of E1A sufficient for posttranslational modification, nuclear-localization, and induction of adenovirus E3 promoter expression in *Xenopus* oocytes

(*E1A* mutants/*Escherichia coli* expression vector/microinjection)

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ABSTRACT **The purified *Escherichia coli*-expressed human subgroup C adenovirus E1A 13S mRNA product induces expression from the adenovirus type 5 E3 promoter when injected into *Xenopus* oocytes. In the present communication, the *E. coli*-expressed E1A 13S and 12S mRNA products are shown to undergo a posttranslational modification in microinjected *Xenopus* oocytes, which causes a 2- to 4-kDa increase in apparent molecular size, identical to that occurring in HeLa cells expressing the *E1A* gene. The *E. coli*-expressed E1A proteins are similarly modified *in vitro* in a soluble rabbit reticulocyte lysate. The modified form of the E1A proteins preferentially localizes to the oocyte nucleus following cytoplasmic microinjection. The use of various deleted forms of E1A protein synthesized in *E. coli* shows that a first exon-encoded domain of E1A, residing between amino acid residues 23 and 120, is sufficient for the posttranslational modification and nuclear localization of E1A and also for the *trans*-activation of the E3 promoter by E1A in *Xenopus* oocytes. These results suggest that the posttranslational modification of E1A protein may be important for its function.**

The adenovirus *E1A* gene encodes products that function in the modulation of gene transcription initiation and in the oncogenic transformation of mammalian cells (1). At early times following viral infection, the human subgroup C adenovirus (adenovirus type 2 and 5) *E1A* gene produces two mRNAs designated 12S and 13S, which result from alternative RNA splicing of the primary transcript (2). The 243-amino acid product of the E1A 12S mRNA differs from the 289-amino acid 13S mRNA product by the internal deletion of 46-amino acid residues (2). We have shown that the human subgroup C adenovirus E1A 13S and 12S mRNA products (E1A 13S and 12S proteins, respectively) produced by plasmid expression vectors in *Escherichia coli* are functional when microinjected into intact cells (3–7).

The E1A 13S and 12S mRNAs each produce two polypeptide products which are resolved by $NaDodSO_4$/PAGE (8–18). Although the mechanism by which the multiple polypeptide species of E1A arise is not known, it has been suggested that this multiplicity is a consequence of posttranslational modification(s) of the primary translation product (12). In this paper we show that the *E. coli*-expressed E1A 13S and 12S proteins indeed undergo a modification when introduced into *Xenopus* oocytes or a soluble rabbit reticulocyte lysate. This modification appears to exactly mimic that which occurs *in vivo*, causing an apparent molecular size increase of about 2 to 4 kDa. We have also examined the ability of a series of *E. coli*-expressed deletion mutant E1A proteins to undergo this posttranslational modification. In addition, we monitored the ability of these deleted forms of E1A proteins to localize in the nucleus and to induce *E3* promoter function following microinjection into oocytes. Our results indicate that a first exon-encoded domain of E1A, between amino acids 23 and 120, contains information sufficient for posttranslational modification and slow nuclear accumulation and for the *trans*-activation of the *E3* promoter in *Xenopus* oocytes.

MATERIALS AND METHODS

Expression in *E. coli* and Isolation of *E1A* Insertion and Deletion Mutant Proteins. We described the construction of pAS1-E1A410 (3) and pAS1-E1A412 (6), which encode and express in *E. coli* the human subgroup C adenovirus E1A 13S and 12S mRNA products, respectively. The construction of derivatives of pAS1-E1A410 that encode E1A deletion mutant proteins is detailed elsewhere (7). The expression in *E. coli* of the E1A-derived proteins was controlled as described (19). The E1A-derived proteins were purified from *E. coli* as described (5). E1A protein concentration was determined by Coomassie blue staining of $NaDodSO_4$/PAGE gels, using bovine serum albumin as a standard. A control protein extract was prepared from *E. coli* containing the plasmid vector pAS1 by using the same protocol used for the purification of E1A proteins.

***Xenopus* Oocyte Microinjection, Fractionation, and Chloramphenicol Acetyltransferase Assay.** *Xenopus laevis* stage VI oocytes were cultured and microinjected by using procedures described (3, 20). For some experiments, 0.5 ng of plasmid pKCAT23 DNA (21) in 10 nl of sterile water was injected into the nucleus (germinal vesicle) of each oocyte. Depending on the experiment, 10 or 50 nl of a solution of E1A-derived protein or control protein [1–3 mg/ml in 20 mM Tris·HCl (pH 7.5 at 25°C), 20 mM KCl, 1 mM EDTA, 0.1 mM dithiothreitol] was injected into the oocyte cytoplasm. Following injection, the oocytes were cultured at 19°C in Barth's medium (34) prior to analysis. Procedures used for the quantitation of chloramphenicol acetyltransferase (CAT) expressed in pKCAT23-injected oocyte have been described (3, 20). *Xenopus* oocytes were fractionated into nuclear and cytoplasmic fractions by manual enucleation. Nuclei were suspended in $NaDodSO_4$/PAGE loading buffer, prior to immunoblot analysis. Whole oocytes or cytoplasmic fractions were prepared by Dounce homogenization in 10 mM Tris·HCl (pH 7.5 at 25°C), 50 mM NaCl and 0.5 mM phenylmethyl-

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Abbreviation: CAT, chloramphenicol acetyltransferase.

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sulfonyl fluoride (100 μl/oocyte) and centrifugation at 5000 × *g* for 5 min. The supernatant protein was precipitated with 4 vol of ethanol, centrifuged (10,000 × *g* for 20 min), and suspended in $NaDodSO_4$/PAGE loading buffer, prior to immunoblot analysis.

Other Procedures. Immunoblot analysis using rabbit antiserum specific to the *E. coli*-expressed E1A 13S mRNA product was carried out as described (5). Procedures used for adenovirus growth and for HeLa cell growth, infection, and transfection have been described (21). A rabbit reticulocyte lysate (Bethesda Research Laboratories) was used according to the supplier's instructions.

RESULTS

Posttranslational Modification of E1A 13S and 12S Proteins. Reports have indicated that the E1A 13S and 12S mRNAs each produce two major polypeptides resolved by $NaDodSO_4$/PAGE in adenovirus-infected cells (8–18). We confirmed this conclusion by E1A-specific immunoblot analysis of HeLa cells transfected with mutant *E1A* genes that express either 13S mRNA or 12S mRNA alone (Fig. 1*A*). The *E. coli*-expressed E1A 13S and 12S proteins have the same $NaDodSO_4$/PAGE mobilities as the faster migrating forms detected in HeLa cells (Fig. 1*A*). These results suggest that the lower apparent molecular weight forms of E1A 13S and 12S protein are the full-length precursor translation products, and the higher molecular weight proteins are posttranslationally modified forms of these precursors.

Using purified *E. coli*-expressed E1A proteins as precursors, we tested whether *Xenopus* oocytes are able to posttranslationally modify the E1A protein. The $NaDodSO_4$/PAGE mobility of both the E1A 13S and 12S proteins is shifted following microinjection into oocytes to precisely the same position as the higher molecular weight form of E1A expressed in HeLa cells (Fig. 1*B*). The *E. coli*-expressed E1A 13S protein is also modified following microinjection into Vero monkey kidney cells (data not shown). These results indicate that the *E. coli*-expressed E1A proteins are indeed precursors for some type of modification and that a similar posttranslational structural alteration of E1A occurs in both *Xenopus* oocytes and mammalian cells.

We examined whether the *E. coli*-expressed protein could also be processed to a form of higher apparent molecular weight in a rabbit reticulocyte lysate, since reports have shown that multiple species of E1A protein arise when E1A mRNA is translated in this system (8–10, 14, 15, 18). Incubation of the *E. coli*-expressed E1A 12S protein (Fig. 1*C*) and 13S protein (data not shown) in the lysate does result in a modification that causes the same shift in $NaDodSO_4$/PAGE mobility (corresponding to an increase in apparent molecular size of 2 to 4 kDa) seen in intact cells.

Nuclear Accumulation and Activation of E3 Promoter Expression by E1A 13S and 12S Proteins. The intracellular distribution of E1A 13S and 12S proteins in injected *Xenopus* oocytes was analyzed by E1A-specific immunoblot analysis of cytoplasmic and nuclear fractions. The modified form of both the E1A 13S and 12S protein is preferentially localized to the oocyte nucleus following cytoplasmic injection (Fig. 2). In addition, both the E1A proteins are modified in enucleated oocytes (data not shown). We point out that the E1A 13S and 12S proteins exhibit a relatively slow rate of accumulation in the oocyte nucleus and even at 5 to 8 hr after injection about half of the E1A protein in the oocyte is still cytoplasmic (Fig. 2*B*). However, by 24 hr after injection >90% of the E1A 13S and 12S protein is localized in the nucleus (Table 1). This is in dramatic contrast to what has been observed in somatic cells where E1A rapidly accumulates in the cell nucleus within 15 to 30 min (5, 6).

We compared the ability of the E1A 13S and 12S proteins to induce expression of the adenovirus 5 E3 promoter in *Xenopus* oocytes. In this assay, the level of CAT enzyme activity is measured in oocytes following nuclear microinjection of a chimeric gene (*E3-CAT*) containing the *E3* promoter linked to the *CAT*-coding sequence and cytoplasmic microinjection of E1A protein or control protein (3). The E1A 12S protein, like the E1A 13S protein (3), is clearly able to stimulate E3-CAT expression (by a factor of 5- to 6-fold) in *Xenopus* oocytes (Table 2).

Posttranslational Modification and Intracellular Localization of Insertion and Deletion Mutant E1A Proteins. The expression in *E. coli* and isolation of a series of deletion mutant *E1A* gene products has been described (Fig. 3) (7). The posttranslational modification of these mutant E1A proteins was monitored by analyzing their $NaDodSO_4$/PAGE mobilities following microinjection into *Xenopus* oocytes (Fig. 4*A*). The results show that the E1A protein undergoes modification in oocytes even after deletion of 21-amino-terminal and/or 67-carboxyl-terminal amino acid residues (products of pAS1-E1A610, 420, and 620). More-

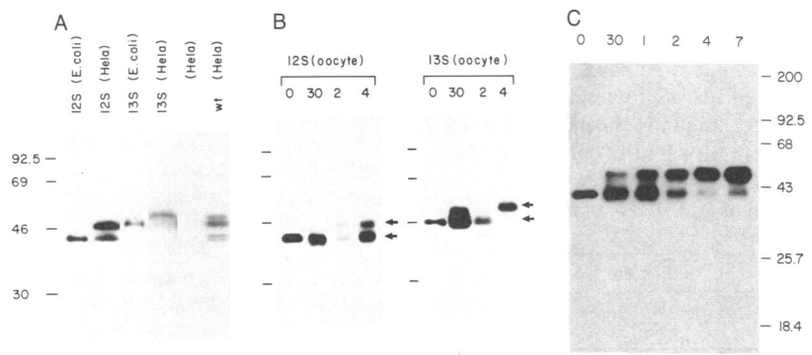

FIG. 1. $NaDodSO_4$/PAGE mobility of E1A proteins expressed in HeLa cells and *E. coli*-expressed E1A proteins injected into *Xenopus* oocytes or incubated in a reticulocyte lysate. (*A*) HeLa cells were transfected with ≈10 μg of pJF12 (12S) or of pJN20 (13S) per 10^6 cells, or were infected with 50 plaque-forming units per cell of wild-type adenovirus type 5 and maintained in the presence of arabinonucleoside (wt). pJF12 and pJN20 expressed E1A 12S mRNA or 13S mRNA, respectively, because part of the *E1A* genomic DNA sequence has been replaced by the corresponding cDNA sequence (ref. 3; N.C.J., unpublished data). *E. coli*-expressed E1A 13S and 12S proteins (2 ng) and total HeLa cell extract (1–10 × 10^5 cells) were analyzed by $NaDodSO_4$/PAGE and E1A-specific immunoblot. HeLa cells were analyzed 40 hr after transfection or 40 hr after infection. (*B*) Purified E1A 13S or 12S protein (20 ng) was microinjected into each *Xenopus* oocyte cytoplasm, and total oocyte extracts were prepared at 0.5, 2, and 4 hr after injection. Noninjected E1A protein (3 ng) (0) and oocyte extracts were analyzed as described above. (*C*) Structural modification of *E. coli*-expressed E1A 12S protein in a rabbit reticulocyte lysate. E1A 12S protein (1 μg) was added to a rabbit reticulocyte lysate (50 μl) and incubated for 0, 0.5, 1, 2, 4, and 7 hr at 30°C. An aliquot of the reaction was analyzed by $NaDodSO_4$/PAGE and E1A-specific immunoblot. The position and size (in kDa) of marker proteins (Amersham) are indicated.

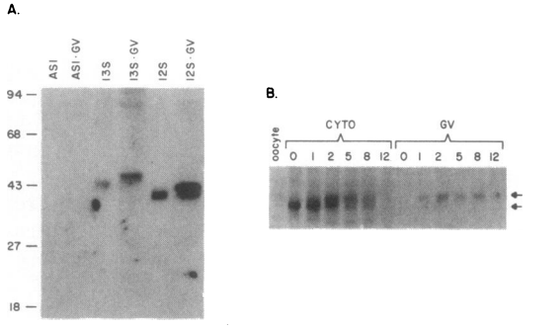

FIG. 2. Modification and intracellular localization of E1A 13S and 12S mRNA products in microinjected *Xenopus* oocytes. (*A*) The oocyte cytoplasm was injected with 20 nl of 1 mg of *E. coli*-expressed E1A 13S protein (13S.GV) or E1A 12S protein (12S.GV) per ml. At 4 hr after injection the oocyte nuclei were isolated manually and analyzed by $NaDodSO_4$/PAGE and E1A-specific immunoblot. Samples of 2–5 ng of noninjected E1A 13S (13S) and 12S (12S) protein were analyzed similarly. Control oocytes were injected with a sample prepared from pAS1-containing *E. coli* and analyzed as described above (AS1, AS1.GV). The position and size (in kDa) of marker proteins are indicated. (*B*) Intracellular distribution of E1A 13S protein as a function of time following cytoplasmic injection into oocytes. Oocytes were separated into cytoplasmic (CYTO) and nuclear (GV) fractions at 0, 1, 2, 5, 8, and 12 hr following cytoplasmic injection with 20 nl of 1 mg of E1A 13S protein/ml and analyzed by $NaDodSO_4$/PAGE and E1A-specific immunoblot. Total oocyte extract was analyzed similarly (oocyte).

over, the entire region encoded by the second exon of *E1A* (product of pAS1-E1A410X) can be deleted, and the protein still undergoes a shift in $NaDodSO_4$/PAGE mobility following oocyte injection. The E1A protein is also modified in oocytes when the deletion extends further within the region encoded by the 3′ end of the first exon of the 12S mRNA (products of pAS1-E1A410 CX and 410C). In contrast, the product of pAS1-E1A410ΔPS, in which most of the amino acid residues of E1A encoded by the 12S mRNA first exon (sequences between the *Pvu* II and *Sma* I sites) are deleted, does not undergo a shift in $NaDodSO_4$/PAGE mobility following microinjection into the oocyte (Fig. 4*A*). These results indicate that a region between amino acids 23 and 120 (encoded between the *Pvu* II and *Cla* I restriction sites within the 12S mRNA first exon of *E1A*) encodes a region sufficient for the posttranslational modification of E1A protein.

We also examined the intracellular distribution of the deleted forms of E1A protein following injection into the oocyte cytoplasm. Table 1 shows that the E1A-derived products of pAS1-E1A610, 620, 420, and 410X are concentrated in the nucleus of oocytes at 24 hr following cytoplasmic injection. Furthermore, it is predominantly the modified form of each of these mutant E1A proteins that is detected in the

Table 1. Intracellular distribution of E1A-derived proteins in microinjected *Xenopus* oocytes

Injected protein	% Nuclear
410	97
412	93
610	85
620	95
420	90
410X	76

Each *Xenopus* oocyte cytoplasm was microinjected with 20 nl containing 1 to 3 mg of various purified E1A-derived protein per ml (Fig. 3). At 24 hr after injection, nuclear and cytoplasmic fractions of the injected oocytes were analyzed by E1A-specific immunoblot. The relative amount of E1A protein in the cytoplasmic and nuclear fractions was quantitated by densitometric scanning of autoradiographs of the immunoblot.

Table 2. Relative CAT activity in *Xenopus* oocytes injected with the *E3-CAT* gene and E1A-derived protein

Protein injected	Relative CAT activity						
	1	2	3	4	5	6	7
None (buffer)	1	1	1	1	1	1	1
AS1	1	1					
410	5	6	3.5	4	4	6	4
412	5	6					
610	6	5					
420	6	5					
620	6	6					
410X			3.5				
410ΔCX				4	5		
410C						4	3.5
410ΔPS						1	1.5

Xenopus oocytes were microinjected with the *E3-CAT* gene and subsequently injected with a solution containing 1–3 mg of E1A-derived protein per ml (Fig. 3), buffer, or an extract prepared from pAS1-containing *E. coli*. At 24 hr after injection, CAT activity in oocyte extracts was assayed, and the CAT activity relative to that in assays with buffer alone was calculated. Oocytes were prepared from different frogs for each of the seven different experiments.

oocyte nucleus (Fig. 4*B*; data not shown). In contrast, the product of pAS1-E1A410ΔPS could not be detected in the oocyte nucleus following cytoplasmic microinjection. These results suggest that the posttranslationally modified region between amino acids 23 and 120 of the E1A protein is important for the accumulation of E1A in the oocyte nucleus.

Induction of *E3-CAT* Expression by Insertion and Deletion Mutant E1A Proteins. The ability of each of the insertion and deletion mutant E1A products to induce expression from the adenovirus *E3* promoter in *Xenopus* oocytes was also examined. Each of the insertion and deletion mutant E1A proteins containing amino acid residues 23–120 (see Fig. 3) stimulated *E3-CAT* expression by 3.5- to 6-fold (Table 2). When amino acid residues 23–120 are deleted, as in the product of pAS1-E1A410ΔPS, no stimulation of *E3-CAT* expression was observed. These results indicate that the first exon-encoded region between amino acid 23 and 120 is an essential domain of E1A required for the stimulation of transcription from the *E3* promoter.

DISCUSSION

We have demonstrated that the *E. coli*-expressed E1A 13S and 12S proteins both undergo a structural modification following microinjection into *Xenopus* oocytes or incubation in a soluble rabbit reticulocyte lysate. This posttranslational modification of E1A causes a $NaDodSO_4$/PAGE mobility shift which corresponds to an increase in apparent molecular size of about 2 to 4 kDa. The $NaDodSO_4$/PAGE mobility of the precursor and modified forms of either the *E. coli*-expressed E1A 13S or 12S protein are identical to the two major products expressed from each E1A mRNA in HeLa cells containing the *E1A* gene. These results establish that of the two major polypeptides resolved by $NaDodSO_4$/PAGE that are encoded by each of the E1A mRNAs, the faster migrating form of E1A is a precursor form, which can be made in *E. coli*, and the slower migrating form is a posttranslationally modified form of this precursor. The nature of this posttranslational modification or structural alteration of E1A is not yet known, but it either may result from covalent modification of the protein or may reflect a conformational transition in the protein. Whatever the nature of this alteration, it clearly depends on and is effectively catalyzed by factors specific to the eukaryotic cell. Our results demonstrate that an *E. coli*-produced protein can

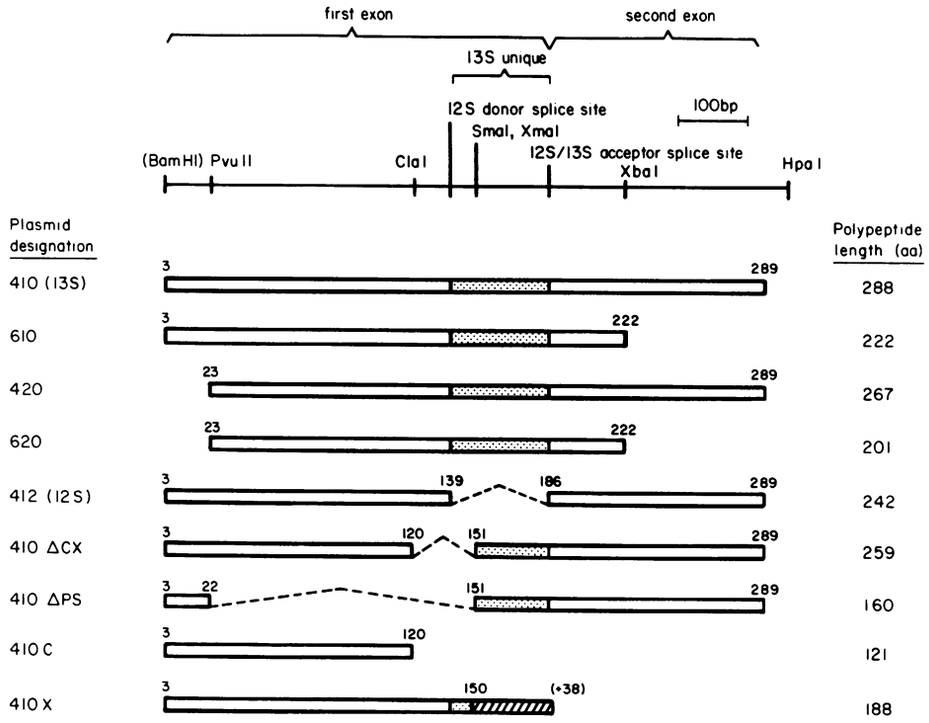

FIG. 3. Schematic representation of the E1A 13S and 12S mRNA products and deletion mutant E1A gene products expressed in *E. coli*. Plasmid expression vectors encoding insertion and deletion mutant E1A proteins were constructed. The number of the first and last amino acid encoded by segments of E1A coding sequence (thick line) that were fused directly to the translation initiation codon supplied by the expression vector (3) are indicated (the authentic E1A 13S mRNA product contains 289 amino acids). 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*.

serve as a substrate to study *in vitro* the posttranslational modification or maturation of a eukaryotic gene product.

In the microinjected *Xenopus* oocyte, the modification of E1A occurs in the cytoplasm, and this processed form of E1A 13S or 12S protein preferentially accumulates in the nucleus. The E1A proteins accumulate in the oocyte nucleus despite the fact that the oocyte cytoplasmic volume is 10- to 20-fold greater than that of the nucleus (22). In general, the rate of nuclear 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 (23). Thus, it appears that the structural modification of E1A protein increases its rate of translocation across the nuclear membrane and/or its binding affinity for some nuclear component.

We used a series of *E. coli*-expressed E1A deletion mutants to map regions of E1A protein that are important for the posttranslational structural modification, nuclear accumulation, and transcription inducing activity of E1A in *Xenopus* oocytes. We find that the same first exon-encoded region, including amino acid residues 23–120, is sufficient for the posttranslational modification and nuclear accumulation and is essential for the induction of the adenovirus *E3* promoter in oocytes. Our results are consistent with and extend a previous analysis of adenovirus mutants that showed that the amino-

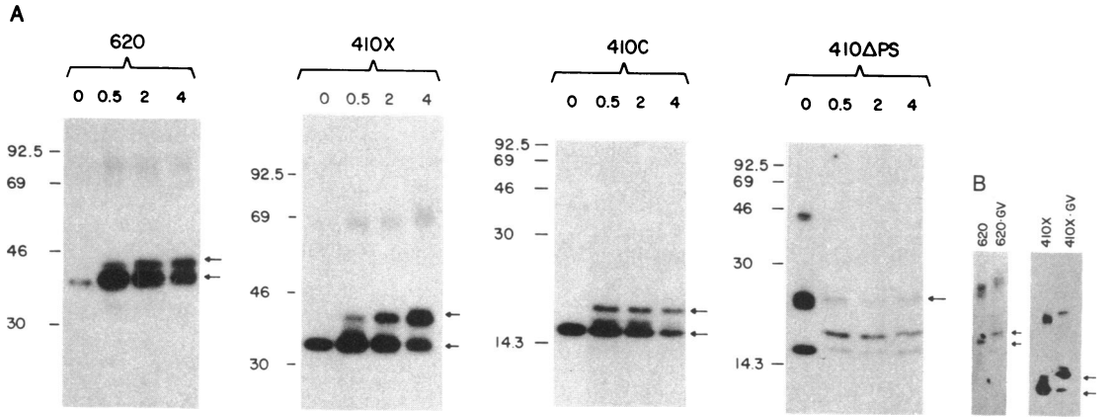

FIG. 4. Modification and intracellular localization of insertion and deletion mutant E1A proteins in microinjected *Xenopus* oocytes. The oocyte cytoplasm was injected with 30 nl containing 1 mg of the purified *E. coli*-expressed product of pAS1-E1A620 (620), 410X (410X), 410C (410C), or 410ΔPS (410ΔPS) per ml. (*A*) Total oocyte extracts were prepared following incubation of the injected oocytes for 0.5, 2, and 4 hr and were analyzed by $NaDodSO_4$/PAGE and E1A-specific immunoblot. A sample of noninjected E1A-derived protein was similarly analyzed (0). The position and size in kDa of protein markers (Amersham) is shown. (*B*) At 4 hr after injection, oocyte nuclei were isolated and were analyzed as described above (620.GV, 410X.GV). A sample of noninjected E1A-derived protein was similarly analyzed (620, 410X).

terminal-15 amino acids and the carboxyl-terminal-70 amino acids of E1A were not essential for the generation of multiple forms of the E1A products in adenovirus-infected cells (11).

The E1A-derived proteins containing this first exon-encoded region do not show major differences in their rates of accumulation in the oocyte nucleus. These results contrast markedly with results obtained when these same preparations of E1A-derived proteins are microinjected into mammalian somatic cells. The rate of accumulation of the E1A protein in the oocyte nucleus is much slower than the rapid rate of nuclear accumulation of E1A protein in mammalian somatic cells. In monkey kidney cells (4–6), E1A protein is completely nuclear localized within 15 to 30 min after cytoplasmic injection. Moreover, the carboxyl-terminal region of the E1A protein is essential for this rapid nuclear accumulation of E1A in monkey kidney cells (7). The E1A carboxyl terminus, however, does not appear to affect the rate of nuclear accumulation of E1A in *Xenopus* oocytes, indicating that this signal-mediated nuclear transport mechanism is not functional in oocytes. The reduced rate of nuclear accumulation of E1A in *Xenopus* oocytes as compared with mammalian somatic cells may reflect differences in the mechanism of nuclear accumulation and/or the availability of certain specific factors important for nuclear localization, as well as differences in cell size. In fact, the rate of nuclear accumulation of E1A 13S and 12S proteins in *Xenopus* oocytes is comparable to the rate of nuclear accumulation in monkey kidney cells of mutant E1A proteins lacking an intact carboxyl terminus. This relatively slow rate of nuclear accumulation (in either oocytes or somatic cells) likely results from simple diffusion of proteins into the nucleus, since the purified E1A 13S and 12S proteins are monomers (unpublished data) and, therefore, small enough to enter the nucleus by diffusion (24). Since the carboxyl-terminal region of E1A protein is the critical determinant for rapid nuclear localization in somatic cells, it is possible that the modification of E1A protein affects its retention in the nucleus rather than its rate of translocation across the nuclear membrane. The modification of E1A may increase its nuclear retention by increasing its affinity for some nuclear component, thus resulting in the selective accumulation of the modified form of E1A protein in the oocyte nucleus. This interpretation is supported by the fact that the site of modification maps to a region of the protein shown to be important for E1A function, i.e., the interaction of E1A with nuclear factors.

The present results indicate that the first exon of E1A encodes a region sufficient to induce expression of the adenovirus E3 promoter in *Xenopus* oocytes. Reports have also indicated that the first exon of E1A may encode a distinct functional domain (25–27). However, it should be emphasized that most evidence suggests that in adenovirus-infected cells, efficient induction of all the early viral genes requires sequences within both exons of the 13S mRNA.

The ability of these same preparations of deletion mutant E1A proteins to complement the E1A-deficient adenovirus 5 mutant *dl*312 was analyzed in monkey kidney (Vero) cells (7). Differences are apparent between the *dl*312 induction assay and the results obtained here with the *E3-CAT* gene in injected *Xenopus* oocytes. The products of pAS1-E1A610, 420, and 620 are able to induce *dl*312 expression with a similar efficiency as the full-length 13S and 12S proteins; these results, therefore, are consistent with their observed ability to induce *E3-CAT* expression in oocytes. The products of pAS1-E1A410X and 410ΔCX induce *dl*312 expression but at 10- to 20-fold lower levels relative to the 13S protein; this reduced efficiency is not observed in the oocyte system. The most striking difference, however, is with the product of pAS1-E1A410C. Although this product induces expression of *E3-CAT* in injected oocytes, it is unable to induce *dl*312 expression (7). This difference probably reflects the fact that *dl*312 complementation assay is a more stringent assay of E1A function since all of the early adenovirus promoters need to be induced to levels sufficient to allow viral replication and subsequent expression from the major late transcription unit. However, cell type differences may also play a role and contribute to the different activities seen.

It is interesting that the c-*myc* (28–31), c-*fos* (32), and *p53* (33) oncogene products, which are also nuclear-localized, occur in multiple forms resolved by $NaDodSO_4$/PAGE. Perhaps the E1A, c-fos, c-myc, and p53 proteins undergo similar posttranslational modifications in mammalian cells.

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