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Induction of Gene Expression by Exon 2 of the Major E1A Proteins of Adenovirus Type 5

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We have constructed an adenovirus type 5 (Ad5) E1A mutant, *dl*1119/520, that produces essentially only exon 2 of the major E1A proteins. In infected primary baby rat kidney cells, this mutant induced expression of the E1B 55-kDa protein, and in infected human KB cells, it induced expression of this protein, the E2A 72-kDa protein, and hexon. In KB cells, this mutant grew substantially better than Ad5 *dl*312, which lacks E1A, and as well as Ad5 *dl*520, an E1A mutant producing only the 243-residue protein. These results suggest that exon 2 of E1A proteins on its own was able to activate gene expression. We also constructed mutants of *dl*1119/520, containing small deletions in regions of exon 2 that others found to be associated with effects on the properties of E1A transformants. None of these deletions destroyed gene activation completely, indicating that there may be some redundancy among sequences in exon 2 for inducing gene expression. The two deletions that decreased induction the most, residues 224 to 238 and 255 to 270, were in regions reported to be associated with the expression of a metalloprotease and with enhanced transformation, suggesting that exon 2 may regulate expression of genes governing cell growth. It is remarkable that all sections of E1A proteins, exon 1, the unique region, and exon 2, have now been found to affect gene expression.

The E1A region of adenovirus type 5 (Ad5) produces two major proteins of 289 and 243 residues (289R and 243R). These products can be thought of functionally in terms of three separate and distinct regions (Fig. 1): exon 1 of the 243R protein, also present in the 289R product; exon 2, present in both proteins; and an internal sequence of 46 amino acids that is unique to the 289R protein. This unique region is a potent activator of viral and cellular genes (25). Exon 1 is the only one of the three regions that is essential for transformation of cells by E1A in cooperation with *ras* (30, 34). Exon 1 can also activate gene expression, which it appears to do through regions within it that bind to cellular proteins, pRb, p107 (21), and p300 (8, 19).

The functions of exon 2 have been analyzed less extensively than those of exon 1 and the unique region. Exon 2 is necessary for inducing a growth factor required for immortalization (22, 23, 28) as well as for the induction of Ad5-specific cytotoxic T lymphocytes and Ad5 tumor-specific transplantation antigen (29; see also reference 1). Exon 2 contains elements that act as auxiliary factors in the transactivation of the adenovirus E4 promoter by the 289R E1A protein (2). Several recent studies have suggested that exon 2 affects the metastatic and other properties of E1A transformants. A sequence in exon 2, residues 223 to 245 (numbered according to the 289R protein sequence; Fig. 1, sequence A), represses expression of a metalloprotease (16). Douglas et al. (5) obtained evidence that mutations between residues 256 and 273 (Fig. 1, sequence B) enhance transformation of baby rat kidney (BRK) cells by E1A in cooperation with activated *ras*. Using exon 2 mutants that we constructed, Boyd et al. (3) found that a sequence toward the C-terminal end of exon 2, residues 271 to 284 (Fig. 1, sequence C), bound a 48-kDa cellular protein, designated CtBP. Furthermore, studies on BRK cells transformed by these E1A exon 2 mutants suggested that CtBP may regulate the proliferation, tumorigenicity, and metastatic potential of these transformants (3).

In recent work on the induction of gene expression by the 243R Ad5 E1A protein, we found evidence that exon 2 itself may be able to induce gene expression (19). Here we describe studies that examine induction of gene expression by exon 2 directly and attempt to link it to exon 2 functions affecting cell growth and transformation.

MATERIALS AND METHODS

Cells and Ad5 E1A mutant viruses. Primary baby rat kidney (BRK) cells were prepared and maintained, and human cells of the KB and 293 lines were grown, as described previously (13).

The construction of mutant viruses has been described for *dl*1151 (20) and for *dl*1133 to *dl*1135 (3); *dl*1132 was constructed in the same way as *dl*1133 to *dl*1135. Mutants *dl*1131/520 and *dl*1119/520 were made by combining suitable restriction fragments from plasmids pLE2*dl*1131, pLE2*dl*1119, and pLE2*dl*520 (14, 15) and transferring the recombinant E1A regions from plasmids into *dl*309 by the method of McGrory et al. (17). Similar methods were used with appropriate plasmids to construct mutants of *dl*1119/520 containing deletions *dl*1132 to *dl*1135 and were designated *dl*19/520/32 to *dl*19/520/35. The Ad5 cDNA 9S virus was a gift from Betty Moran (Cold Spring Harbor, N.Y.) (18). Virus titers were determined on 293 cells (9).

Immunoprecipitation of labeled proteins. Cells infected at 30 PFU per cell were labeled from 16 to 20 h postinfection (p.i.) with 100 μCi of [^{35}S]methionine (Dupont-NEN, Boston, Mass.) per 60-mm-diameter dish in methionine-free medium. Lysates were prepared and immunoprecipitated for different proteins as follows: for E1A, as described previously (12); for E2A 72-kDa protein and hexon, as for E1A except that the lysis buffer contained 0.1% sodium dodecyl sulfate (SDS). For E1B 55-kDa, E3 19-kDa, and Hsp90 proteins, total cell extracts were prepared and immunoprecipitated as described previously (4). The monoclonal antibodies used were M58 and M73 (against E1A proteins) and 9C10 (against the E1B 55-kDa

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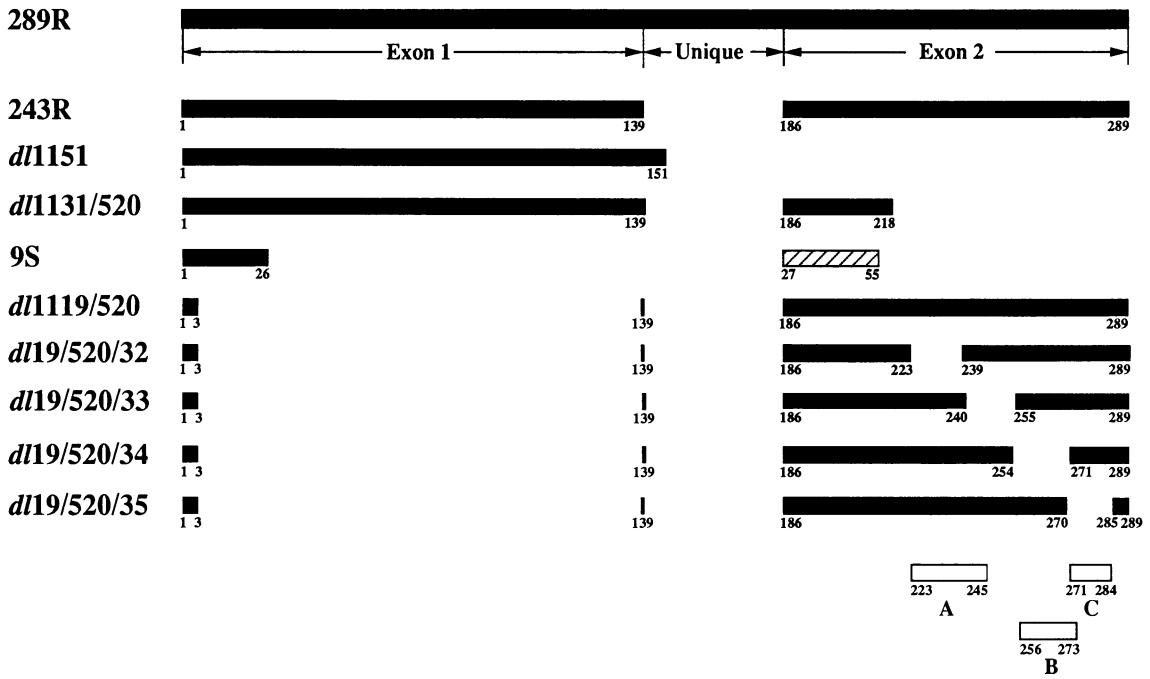

FIG. 1. Map of the 289R and 243R Ad5 E1A proteins, the proteins produced by E1A mutants used in this study, and functional regions in exon 2. In each case, the portions of the 289R E1A protein produced are shown by black bars, together with first and last residues, numbered according to their positions in the 289R protein. In the 9S product, residues 27 to 55, indicated by a hatched bar, are translated from a different reading frame. At the bottom are shown the regions in exon 2 required for repression of a metalloprotease (16) (A), enhanced transformation (5) (B), and binding to CtBP (3) (C).

protein) (Oncogene Science, Uniondale, N.Y.), H2-19 (against the E2A 72-kDa protein; from P. E. Branton [McGill University]), 9F6-6 (against hexon; produced by J. Williams [Carnegie-Mellon University, Pittsburgh, Pa.] and obtained from L. Prevec [McMaster University]), and AC 88 (against Hsp90; Stress-Gen Biotechnologies Corp. [Victoria, British Columbia, Canada]). Antiserum P38, against the E3 19-kDa protein (33), was from W. Wold (Washington University).

Western immunoblots. Cells were infected, harvested at 20 h p.i., and lysed. Lysates were electrophoresed on SDS-polyacrylamide gels containing 12.5% acrylamide. Samples were transferred to nitrocellulose filters by using semidry electrophoresis and blocked (11). Antibodies were applied and detected by using the enhanced chemiluminescence procedure as supplied by Amersham Canada Ltd., Oakville, Ontario, Canada. The primary antibody was a polyclonal antibody to Nm23 peptide 11 (24) from P. S. Steeg (National Institutes of Health, Bethesda, Md.).

RESULTS

To test the capacity of exon 2 of E1A proteins to activate gene expression on its own, we constructed E1A mutant virus *dl*1119/520 (Fig. 1). This construct combines *dl*1119, which is deleted of residues 4 to 138 from exon 1 (14), with *dl*520, a mutation that allows the production of the 243R but not the 289R protein from E1A (10). As a result, *dl*1119/520 makes only a severely deleted form of the 243R protein, consisting of residues 1 to 3 and 139 of exon 1, together with all of exon 2.

We compared the abilities to activate gene expression of this mutant with those of the following viruses (Fig. 1): *dl*309, producing 289R and 243R E1A proteins; *dl*520, producing only the 243R E1A protein; *dl*1151, producing a single E1A polypeptide consisting of the N-terminal 150 residues of the 289R protein, i.e., exon 1, with 11 residues of the unique region; *dl*1131/520, which combines *dl*520 with *pm*1131 (14) so as to produce a 243R protein that lacks the C-terminal 71 residues of exon 2; *dl*312, lacking essentially all of E1A; and a 9S cDNA virus, producing only a 9S E1A mRNA, the product of which theoretically contains only residues 1 to 26 in common with the two major E1A products.

As targets for induction, we tested genes for viral proteins against which antibodies were readily available. We did this by infecting BRK and human KB cells with mutant viruses at 30 PFU per cell, labeling the cells with [^{35}S]methionine from 16 to 20 h p.i., and immunoprecipitating the gene products from cell lysates with appropriate antibodies. The immunoprecipitates were then analyzed by SDS-polyacrylamide gel electrophoresis. Figure 2 shows the results from a single experiment for the E1B 55-kDa protein, the E2A 72-kDa DNA-binding protein (DBP), the E3 19-kDa protein, and hexon. Each mutant was tested at least twice with similar results.

In BRK and KB cells, *dl*309 and *dl*520 induced all of the proteins (Fig. 2, lanes 1 and 2), although in KB cells, *dl*520 was noticeably weaker than *dl*309 for the E1B 55-kDa protein and especially for the E3 19-kDa protein. *dl*1151 and *dl*1131/520 (lanes 3 and 4) induced all of the proteins except the E3 19-kDa protein, but for hexon, induction was weaker than with *dl*520. *dl*1119/520 (lane 5) induced the E1B-55 kDa protein in both BRK and KB cells and the E2A 72-kDa protein and hexon in KB cells but failed to induce E2A DBP in BRK cells or the E3 19-kDa protein in KB cells. The levels of the E1B 55-kDa and E2A 72-kDa proteins induced in KB cells by *dl*1119/520 were comparable to those produced by *dl*520. *dl*312 and 9S virus (lanes 6 and 7) induced only E2A DBP in KB cells

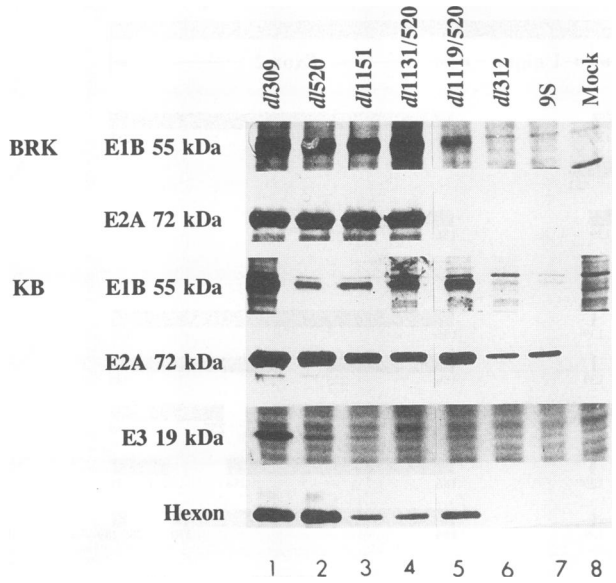

FIG. 2. Induction of gene expression by mutant viruses producing only exon 1 or exon 2 of the 243R protein. ^{35}S-labeled extracts from BRK and KB cells infected with the viruses indicated (lanes 1 to 8) were immunoprecipitated with antisera against the viral proteins shown on the left. The immunoprecipitates were analyzed on SDS-polyacrylamide gels and autoradiographed.

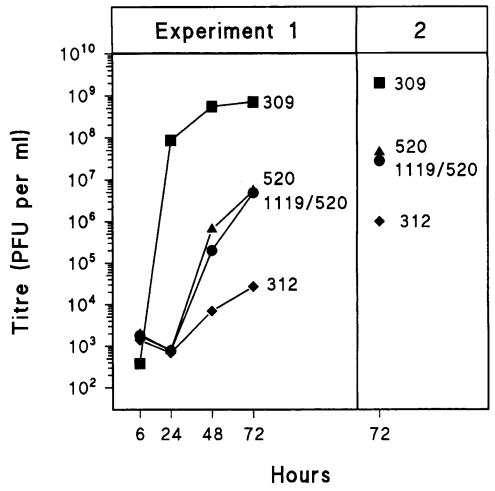

FIG. 3. Growth of E1A mutant viruses on KB cells. In experiment 1, KB cells were harvested and lysed at different times p.i., and the yields of virus in PFU were measured on 293 cells. Experiment 2 was similar except that yields were measured only at 72 h p.i.

at reduced levels. There was no induction of any of the proteins in mock-infected cells (lane 8). Simon et al. (26) reported that adenovirus infection induced Hsp90 in HeLa cells. However, we were unable to examine its induction in KB cells, as we found that it was expressed constitutively in these cells. The results with mutants *dl*1151 and *dl*1119/520 are summarized in Table 1.

In BRK cells, the results with *dl*1151 duplicated those of an earlier study, in which we concluded that regions in exon 1 are necessary and sufficient for expression of the E1B 55-kDa and E2A 72-kDa proteins (19). In this previous work, we obtained evidence that only regions in exon 1 could induce expression of the E2A DBP but that exon 2 may provide an alternative pathway to that of exon 1 for induction of the E1B 55-kDa protein. The results with *dl*1119/520 (Fig. 2) confirmed that exon 2 could not induce E2A DBP in BRK cells but showed that exon 2 alone could induce expression of the E1B 55-kDa protein. In KB cells, both *dl*1151 (exon 1) and *dl*1119/520 (exon 2) induced the three proteins, E1B 55 kDa, E2A 72 kDa, and hexon. Previously we found that a number of mutants, including *dl*1151, induced the E3 19-kDa protein in BRK cells (19).

TABLE 1. Abilities of exons 1 and 2 of E1A to induce viral gene expression in BRK and KB cells

Cell type	Gene	Induction of gene expression	
		*dl*1151 (exon 1)	*dl*1119/520 (exon 2)
BRK	Early viral E1B 55 kDa	+	+
	Early viral E2A 72 kDa	+	−
KB	Early viral E1B 55 kDa	+	+
	Early viral E2A 72 kDa	+	+
	Early viral E3 19 kDa	−	−
	Late viral hexon	+	+

Compared with its induction in BRK cells, this protein was induced at a low level in KB cells, even by *dl*309; the level was lower still with *dl*520, and it was negligible with the other mutants (Fig. 2).

It is evident from these experiments that induction of gene expression by exon 2 varied between genes; for example, in BRK cells, exon 2 induced the E1B 55-kDa protein but not the E2A DBP. Induction also varied between cell types: exon 2 induced E2A DBP and hexon in KB cells, but the evidence here (Table 1) and in a previous study (19) showed that it did not induce these proteins in BRK cells.

Growth of mutant virus *dl*1119/520. As a further test of the ability of E1A exon 2 to function on its own, we measured the growth of *dl*1119/520 in KB cells. KB cells were infected at 2 PFU per cell with *dl*1119/520, *dl*309, *dl*520, or *dl*312 and harvested at different times p.i. The yields of infectious virus were determined on 293 cells (Fig. 3, experiment 1). The experiment was repeated except that the yields of virus were measured only at 72 h p.i. (Fig. 3, experiment 2). Relative to *dl*309, yields with *dl*520 were reduced 50- to 100-fold, compared with the 5-fold reduction reported previously for a virus producing only the 243R E1A protein (32). However, the more significant result was that in both of our experiments, the yield of virus with *dl*1119/520 was essentially identical to that with *dl*520 and significantly greater than that with *dl*312. This finding demonstrated that E1A exon 2 alone induced viral and cellular genes required for viral replication as effectively as the whole 243R protein.

Analysis of mutant E1A and associated proteins. It was surprising that the E1A protein of *dl*1119/520 was sufficiently stable to produce the results described above, despite the extensive deletion that it contained. To examine this protein and the E1A proteins of the other mutants, we infected KB cells at 30 PFU per cell and labeled them with [^{35}S]methionine from 16 to 20 h p.i. From cell lysates, we immunoprecipitated E1A proteins with a mixture of E1A-specific antibodies M58 and M73, which recognize epitopes in exon 1 and exon 2, respectively, and analyzed the immunoprecipitates on SDS-polyacrylamide gels (Fig. 4). High levels of E1A protein were detected not only from *dl*309 and *dl*520 (lanes 1 and 2) but also from *dl*1119/520 (lane 5). Lower levels were detected with

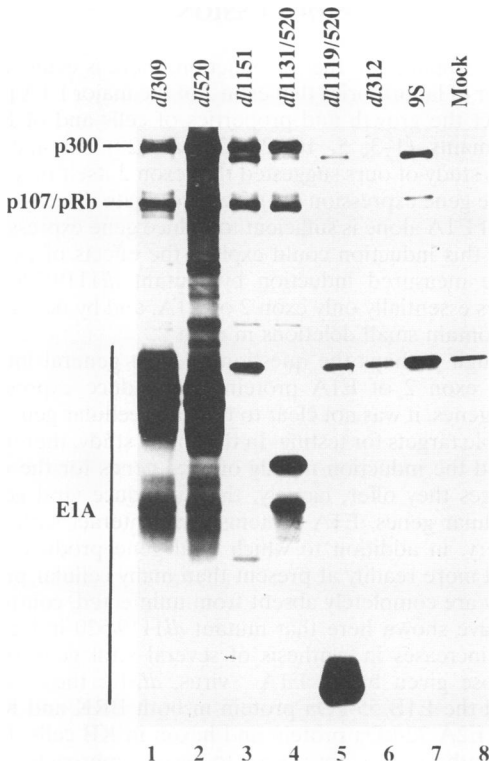

FIG. 4. Fluorograph of an SDS-polyacrylamide gel of anti-E1A immunoprecipitates from ^{35}S-labeled extracts of KB cells, infected with mutant viruses producing only exon 1 or exon 2 of the 243R protein. The infecting virus is indicated for each lane. Bands representing E1A proteins and the cellular proteins p300 and p107/pRb are marked; in this gel, p107 and pRb were not resolved.

*dl*1151 and *dl*1131/520 (lanes 3 and 4), but this may have been partly because the avidity of M58 is less than that of M73. The mobility of the *dl*1119/520 product corresponded to a molecular mass of about 18 kDa.

E1A proteins bind to the cellular proteins p300, p107, and pRb through regions in exon 1 (6, 30, 31). As expected, bands of these proteins were found in the precipitates from *dl*309, *dl*520, *dl*1151, and *dl*1131/520, all of which contain exon 1 (Fig. 4, lanes 1 to 4), but not from *dl*1119/520, which does not (lane 5). Other than E1A, no proteins were detected in cells infected with *dl*1119/520 (lane 5) that were not also seen in cells infected with *dl*312 or 9S virus or in mock-infected cells (lanes 6 to 8). (The pairs of bands at the tops of lanes 5 to 8 migrated at different rates from p300 and p107/pRb and so do not represent these proteins.) Similar results to these were obtained with BRK cells (not shown). In none of these analyses or those of Fig. 6 described below could we detect CtBP (3). This was because the M73 antibody, which was the only one that we could use to precipitate the E1A protein of *dl*1119/520, binds to the same sequence in the E1A protein as does CtBP (7) and probably outcompeted it during immunoprecipitation.

We have found from experience that the cellular proteins to which mutant E1A proteins bind provide an accurate means of confirming the identities of E1A mutant viruses. The patterns in Fig. 4 therefore strongly supported the identities of the mutants that we used in this work. The lack of associated cellular proteins with *dl*1119/520 also showed that this virus was not contaminated with another that contained exon 1 and might otherwise have accounted for the induction of gene expression that we observed.

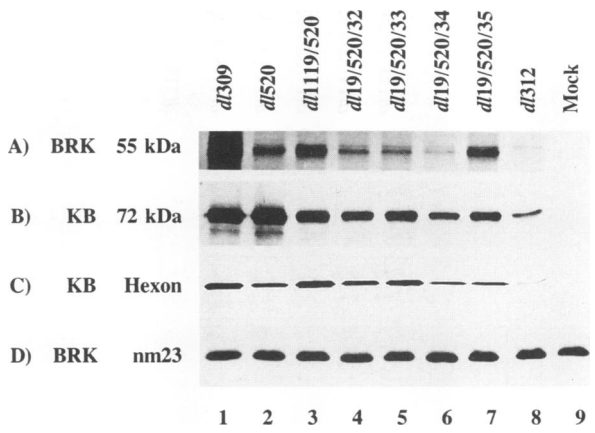

FIG. 5. Induction of gene expression by E1A mutant viruses producing only portions of exon 2. (A to C) Details as for Fig. 2; (D) Western immunoblot of Nm23 protein in quiescent BRK cells infected with mutant viruses (lanes 1 to 8) compared with mock infection (lane 9).

Mapping regions in exon 2 for inducing gene expression. In light of results demonstrating that specific regions in exon 2 repress expression of a metalloprotease (16), modulate cell transformation (5), and bind to CtBP (3) (Fig. 1, sequence A to C), we examined whether deletions in these regions affected the ability of exon 2 to induce gene expression. For this purpose, we built deletions *dl*1132 to *dl*1135 into *dl*1119/520 and tested the resulting viruses (designated *dl*19/520/32 to *dl*19/520/35) (Fig. 1) for their abilities to induce the E1B 55-kDa protein in BRK cells and the E2A 72-kDa protein and hexon in KB cells. Results from a single set of experiments are shown in Fig. 5A to C. Except for hexon, in which case we were limited by the availability of antiserum, all of the experiments were repeated with similar results. Measurements on the induction of the E2A DBP in KB cells were quantified from the radioactivity of the immunoprecipitates, as most of it was in this protein. Table 2 shows the results from two experiments expressed as percentages of the incorporation for *dl*309. Figures 5A to C and Table 2 show that all four deletions, *dl*1132 to *dl*1135, affected the induction of gene expression to different degrees. In all of the experiments with the three different genes, *dl*19/520/34 (Fig. 5A to C, lane 6) consistently gave the weakest induction (see also Table 2). On average, the next weakest was *dl*19/520/32 (lane 4), with *dl*19/520/33 and *dl*19/520/35 (lanes 5 and 7) giving the strongest inductions, except in

TABLE 2. Induction of the E2A 72-kDa DBP in KB cells by E1A mutant viruses

Mutant	Induction (%)	
	Expt 1	Expt 2
*dl*309	100	100
*dl*520	110	88
*dl*1131/520	21	12
*dl*1119/520	38	32
*dl*19/520/32	17	20
*dl*19/520/33	24	26
*dl*19/520/34	12	13
*dl*19/520/35	17	27
*dl*312	4	7

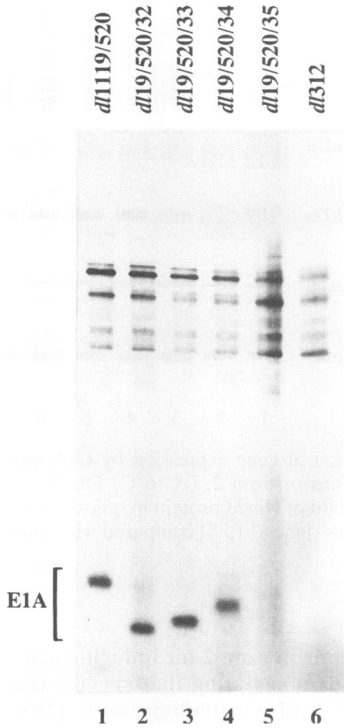

FIG. 6. Fluorograph of an SDS-polyacrylamide gel of immunoprecipitates from ^{35}S-labeled extracts of KB cells infected with mutant viruses producing only portions of exon 2. The infecting virus is indicated for each lane. Bands representing E1A proteins are marked.

the single experiment with hexon, in which case *dl*19/520/35 was weaker than *dl*19/520/32.

Boyd et al. (3) found that *dl*1135 increased the tumorigenic and metastatic potential of cells transformed by E1A in cooperation with *ras*. Earlier, Steeg et al. (27) had reported that E1A activated expression of *NM23*, a gene that these authors had previously found suppressed metastatic progression. To explain their own observations, Boyd et al. (3) suggested that E1A may activate *NM23* by binding to CtBP through exon 2 and that this activation would be lost in *dl*1135, which fails to bind to CtBP. To examine this possibility, we infected KB and quiescent BRK cells with exon 2 mutants at 30 PFU per cell and harvested them at 20 h p.i. Western immunoblots were made by using an antibody to a peptide of the *NM23* product. In both KB and BRK cells, we found that the Nm23 protein was expressed constitutively and that expression was not affected by infection with any of the viruses. The results of an experiment with BRK cells are shown in Fig. 5D; the band detected in these blots migrated at a rate corresponding to a molecular mass of about 19 kDa, similar to that reported for Nm23 protein in human cells (24).

We analyzed the E1A proteins from the exon 2 mutants after immunoprecipitating them from lysates of infected cells with M73 antiserum (Fig. 6). Bands of similar intensities, migrating faster than that with *dl*1119/520 (lane 1), were seen with *dl*19/520/32 to *dl*19/520/34 (lanes 2 to 4). Differences in levels of induction given by these mutants were therefore not due to differences in levels of E1A proteins. As expected, no protein was seen with *dl*19/520/35 (lane 5), as it lacks the epitope for M73 (7). Nevertheless, this mutant must have produced protein, as it activated expression of all the genes better than some other mutants did (Fig. 5; Table 2).

DISCUSSION

As we explained in the introduction, there is evidence from a number of laboratories that exon 2 of the major E1A proteins can affect the growth and properties of cells and of E1A-*ras* transformants (1–3, 5, 16, 22, 23, 28, 29). In addition, a previous study of ours suggested that exon 2 itself may be able to induce gene expression (19). To investigate directly whether exon 2 of E1A alone is sufficient to induce gene expression and whether this induction could explain the effects of exon 2 on cells, we measured induction by mutant *dl*1119/520, which expresses essentially only exon 2 of E1A, and by derivatives of it that contain small deletions in exon 2.

Although perhaps the question of most general interest is whether exon 2 of E1A proteins can induce expression of cellular genes, it was not clear to us which cellular genes would be suitable targets for testing. In this initial study, therefore, we examined the induction mainly of viral genes for the obvious advantages they offer, namely, that to induce viral genes, as with cellular genes, E1A proteins must interact with cellular machinery, in addition to which viral gene products can be detected more readily at present than many cellular products, and they are completely absent from uninfected, control cells.

We have shown here that mutant *dl*1119/520 induced substantial increases in synthesis of several viral gene products over those given by an E1A$^-$ virus, *dl*312; these products included the E1B 55-kDa protein in both BRK and KB cells and the E2A 72-kDa protein and hexon in KB cells. In some cases, synthesis was increased to levels approaching those given by *dl*520, which produces wild-type 243R E1A protein. However, *dl*1119/520 failed to activate the genes for the E2A 72-kDa protein in BRK cells and the E3 19-kDa protein in KB cells. Thus, induction by E1A exon 2 was not universal but depended both on the individual gene and on the cell type.

Previous work demonstrated that in HeLa cells at early times after infection at 20 or 50 PFU per cell with *dl*312, the amount of viral RNA in the cytoplasm is negligible, and the rate of transcription of early viral genes is barely detectable (15a, 20a). Therefore, to increase synthesis of gene products, E1A exon 2 must increase transcription. Further experiments are required to confirm this and to determine whether the increase results from increased initiation, as seems probable, or from an increased rate of transcription. If E1A exon 2 does affect initiation, the differences that we observed between genes and cell types probably reflect differences in the ability of exon 2 to interact with factors governing initiation that differ between genes and differ between human and rat cells for the same gene.

The preservation of exon 2 in E1A throughout the evolution of adenovirus suggests that it performs an essential task. That this task could be to induce expression of the viral and cellular genes necessary for virus replication is suggested by our observations that in human KB cells, *dl*1119/520 induced expression of the genes for the E1B 55-kDa protein, E2A 72-kDa DBP, and hexon and grew substantially better than *dl*312, a virus wholly lacking E1A. Indeed, our finding that *dl*1119/520 grew as efficiently as *dl*520 in KB cells suggested that exon 2 alone can be as effective as the entire 243R E1A protein for inducing the gene expression required for virus production. However, the reduced levels of virus production with both *dl*1119/520 and *dl*520 compared with *dl*309 confirmed previous observations (32) that the unique region must activate essential genes that exons 1 and 2 do not. Evidently exons 1 and 2 and the unique region serve different, if in some cases overlapping, functions for regulating gene expression.

Tests on the induction of gene expression by derivatives of

*dl*1119/520 containing deletions *dl*1132 to *dl*1135 in exon 2, i.e., *dl*19/520/32 to *dl*19/520/35 (Fig. 1), showed that all of the deletions reduced induction to some extent but that the reductions were greater with *dl*1132 and *dl*1134 than with *dl*1133 and *dl*1135. As none of the deletions abolished induction completely, these tests did not identify any particular region in exon 2 that was absolutely essential for induction. This finding could mean there is some redundancy in exon 2. One possibility is that the regions that we deleted in exon 2 bind to a number of different regulatory proteins in the cell and that the effect of this binding on gene activation is cumulative. Alternatively, much of exon 2 may form a surface for binding a single cellular protein that regulates gene expression. In this case, the small deletions that we made may have altered to different extents the shape of this surface and consequently the strength of binding to this cellular protein.

So far, the only cellular protein that exon 2 is known to bind to and that could account for gene activation is CtBP, and our results (Fig. 5A to C; Table 2) showed that activation had comparatively little requirement for the CtBP binding region (residues 271 to 284, deleted in *dl*19/520/35; Fig. 1, sequence C). Most of the region identified for metalloprotease inhibition (Fig. 1, sequence A) is deleted by *dl*1132, and this deletion caused a noticeable reduction in gene activation (*dl*19/520/32 in Fig. 5A to C and Table 2). However, the best correlation that we observed was with the region between residues 256 and 273 (Fig. 1, sequence B), which Douglas et al. (5) identified as important for modulating transformation of BRK cells by E1A together with activated *ras*. This sequence corresponds closely to that deleted by *dl*1134, residues 255 to 270, and it was this mutant, *dl*19/520/34, that consistently gave the weakest induction with all of the genes that we examined (Fig. 5A to C; Table 2). It is possible therefore that induction of appropriate regulatory proteins in the cell by regions in exon 2 could explain the effects of exon 2 on cells observed by others, and in particular that the regions deleted by *dl*1132 and *dl*1134 induce the synthesis of regulators inhibiting expression of protease genes or genes that enhance transformation. It is apparent that to clarify the effects of exon 2, the mechanism by which it induces gene expression requires much more analysis. Unfortunately, the low levels of induction given by several of the exon 2 mutants may make this difficult.

The results presented in this report demonstrate that exon 2 of E1A can independently induce gene expression. E1A proteins are therefore remarkable for containing a minimum of four regions through which induction can be accomplished: two in exon 1 (19), the unique region, and at least one in exon 2. This plethora of gene regulatory regions underscores the complex roles of E1A proteins in the viral life cycle and in transformation. Studies on the mechanisms governing the use of these different regions and the classes of genes affected by each of them could provide important information on gene regulation in cells.

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