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Adenovirus *E1A* gene autorepression: Revertants of an *E1A* promoter mutation encode altered E1A proteins

(autoregulation of genes/enhancer elements/regulation of transcription/repression/E1A functional domains)

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ABSTRACT **Revertants have been isolated from Ad3hr15, a mutant of human adenovirus type 3 that carries a defective *E1A* promoter. Transcription of these revertant *E1A* genes is restored—from nil for Ad3hr15 mutant to levels exceeding that of the wild-type virus. The mutant Ad3hr15 virus and the revertants all have an aberrant *E1A* promoter that contains two short tandem duplications of viral DNA sequence. The *E1A* gene-coding region of the mutant is the same as that for wild-type adenovirus type 3, whereas the revertants are characterized by short in-frame deletions within the 5′ exon region of their *E1A* genes. Location of these reverting, second-site deletions is discussed in relation to *E1A* gene autoregulation and the evolved diversity of *E1A*-related oncogenic potential among different human adenoviruses.**

Adenovirus *E1A* gene products act in trans to positively and negatively regulate transcription of a variety of viral and cellular genes. Positive and negative controls of transcription also apparently operate in the autoregulation of the adenovirus *E1A* gene (1–3). Specific cis elements in DNA sequences that may confer the *E1A*-activation property upon an arbitrary promoter remain elusive. However, *E1A*-mediated repression of transcription from specific promoters has been clearly associated with transcriptional enhancer elements (4–7). We have recently described Ad3hr15 (8), a defective mutant of human adenovirus type 3 (Ad3), which duplicates an enhancer region ≈200 base pairs (bp) upstream from the transcriptional start site of its otherwise wild-type *E1A* gene. Although wild-type Ad3 generates abundant *E1A* transcripts throughout lytic infection, no detectable *E1A* mRNA was recovered from cells early after abortive infection by the Ad3hr15 mutant. We report here the isolation of phenotypic revertants of Ad3hr15 that restore transcriptional activity of its *E1A* gene to levels above the *E1A* gene expression of wild-type Ad3. These revertants retain aberrant *E1A* promoter of Ad3hr15 but lack short in-frame DNA sequences within the coding region of the 5′ exon of the *E1A* gene. Thus the duplicated enhancer of the Ad3hr15 *E1A* promoter appears to render transcription of the gene exceptionally sensitive to repression by *E1A* products in trans. Deletions in the revertant *E1A* genes define an autorepressive domain within the *E1A* gene products. This region of the *E1A* gene is interesting also with regard to the evolutionary origins and variable tumorigenicity of different subgroups of adenovirus serotypes.

MATERIALS AND METHODS

Cells and Viruses. Cultures of cell line 293 cells, human embryonic kidney cells transformed by DNA of human Ad5, were obtained from T. Shenk (Princeton University). The A549 cell line, derived from a human lung carcinoma and equivalent to HeLa or KB cells for growth of Ad3 or Ad5, came from the American Type Culture Collection. Monolayer cell cultures were maintained in Dulbecco's modified Eagle's medium with 10% calf serum as described (8). Human Ad5, strain Adenoid 6, and Ad3, strain G.B., were originally from the American Type Culture Collection and have been propagated as reported by this laboratory (1, 8, 9–11). Isolation and characterization of the host range, defective mutant Ad3hr15 was described by Larsen *et al.* (8); the mutant can only be propagated by infection of cell line 293 cells and is defective for *E1A* transcription and growth in A549 cells. Virions for isolation of DNA or for inoculation of cell cultures were extracted from infected cells and purified by three consecutive centrifugations in CsCl equilibrium density gradients (Ti75 rotor; 40,000 rpm at 4°C for 16–20 hr). Viral concentrations were estimated by dilution of an aliquot of the preparation in 0.5% sodium lauryl sulfate solution (to lyse virus and reduce light scattering) and determination of OD at 260 nm (1.0 A_{260} unit = 1.0×10^{12} virions per ml).

Isolation of Revertants of Ad3hr15. Purified virions of Ad3hr15 (from infected 293 cell cultures) were used to infect A549 cell cultures at 1250 particles per cell. Cells, debris, and media from this passage of the virus were collected 2 weeks after infection, then sonicated and cleared by low-speed centrifugation at 2000 rpm for 5 min. The supernatant was then used as inoculum for a second passage on fresh cultures of A549 cells. Plaque titration on A549 cells of the cleared lysates from the first and second passages yielded plaques, most of which were similar to those of wild-type Ad3: 0.5–5.0 mm in diameter at 10–14 days after infection. At low dilutions of the first lysate, a background of pinpoint-sized microplaques appeared, typical of local nonproductive effects seen in high multiplicity infections of A549 cells by Ad3hr15 (8). The second lysate showed few microplaques at low dilution; however, several exceptionally large plaques (exceeding 10 mm in diameter) atypical of wild-type Ad3 did appear. In each lysate the numbers of the wild-type-sized and the large plaques were proportional to dilution, suggesting single-hit infection kinetics. Forty-five independent revertant plaques were picked from the first and second lysate infections for the further characterization described in this report. Passage of these revertant viruses on A549 cells led to rapid, adenovirus-type cytopathic effects and yielded large quantities of virus.

DNA, Enzymes, and Restriction/Sequence Analysis. Restriction enzymes and other enzymes for DNA analysis were obtained from New England Biolabs and used as directed by the supplier. DNA fragments of restriction digests were separated by electrophoresis in 1% agarose slab gels with 0.5 μg/ml ethidium bromide for detection of DNA by fluores-

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Abbreviations: Ad, adenovirus type; hr, host range, as for the defective mutant Ad3hr15; *E1A*, the gene that constitutes the *A* portion of early region 1 of the adenovirus genome.

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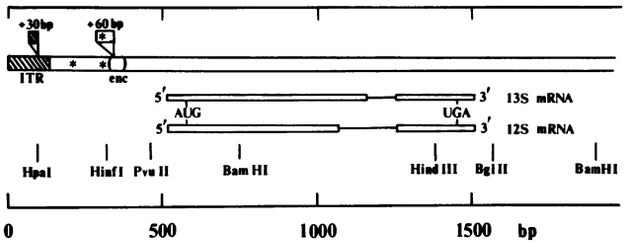

FIG. 1. Physical map of the left-end *E1A* region of Ad3 and mutant Ad3hr15. The left 2000 bp of the viral genome is represented at the top of the figure with the left-end 135-bp inverted terminal repetition (ITR) crosshatched. Asterisks (*) correspond to the duplicated *E1A* enhancer element (located 200 and 300 bp upstream from the *E1A* transcription start site) described in Ad5 by Hearing and Shenk (13). The region required for cis-polar DNA encapsidation (enc) of Ad3 is shown adjacent to the "−200" promoter-proximal enhancer element. The *E1A* region of Ad3hr15 differs from wild-type Ad3 by two tandem DNA sequence reiterations, shown as inserts above the Ad3 DNA map. Structures corresponding to the early 12S and 13S mRNA species of Ad3 (and Ad3hr15) are shown below the DNA map. Introns appear as single lines between the 5′ and 3′ exons of the mRNA. Genomic landmarks of the Ad3 mRNA structures are specified: the 5′ cap site (bp 511); 13S intron (bp 1155–1249, inclusive); 12S intron (bp 1062–1249, inclusive); translation start and stop codons (ATG, bp 575; TGA, bp 1453); and 3′ polyadenylylation site (bp 1511). The sequence of the Ad3 *E1A* gene was determined by Robert Hall of this laboratory (unpublished work) and is 98% identical to the published sequence of the closely related Ad7 *E1A* gene. Positions of DNA restriction sites are shown below the mRNA and DNA structures.

cence. DNA fragments (*Hpa* I, bp 95–*Bgl* II, bp 1565) from the *E1A* genes of the revertant viruses were subcloned into the pEMBL 18 plasmid vector before determining DNA sequence. Recombinant DNA was cleaved with *Bam*HI, then 3′ end-labeled using [α-^{32}P]dCTP together with unlabeled dATP, dGTP, dTTP and *Escherichia coli* DNA polymerase I, Klenow fragment. *Hin*dIII was used for secondary restriction to segregate the labeled ends before proceeding with the chemical-cleavage protocols of Maxam–Gilbert (12).

RNA Blot Analysis of RNA from Infected Cells. Cultures of A549 or 293 cells were infected with freshly prepared, 3× CsCl-banded adenovirus at 2000 particles per cell. Coinfections with two different adenoviruses were inoculated at 1000 particles of each viral type per cell. RNA was extracted at 3, 6, 9, and 20 hr after infection by use of guanidinium thiocyanate and centrifuged through a 5.7 M CsCl cushion (SW41 rotor, 27,000 rpm at 15°C for 16 hr). RNA hybridization analysis was done after electrophoresis of RNA samples (10 μg of RNA per track) in formaldehyde-agarose gels. The gels were blotted to GeneScreen (DuPont), hybridized with nick-translated plasmid DNA bearing Ad5 or Ad3 *E1A* genes, and then exposed for autoradiography. Details of the entire procedure, including descriptions of Ad3 and Ad5 *E1A*-specific DNA probes have been reported (1).

RESULTS

Revertants of Ad3hr15. The physical map of the Ad3 *E1A* gene (Fig. 1) is very similar to maps of the more familiar subgroup C adenoviruses Ad2 and Ad5. The early *E1A* gene transcripts differ only by the length of intron removed. Their translation products share common amino- and carboxy-terminal peptide sequences. The defective mutant Ad3hr15 has two short tandem DNA repetitions, shown as inserts above the genome map. The left duplication (bp 69–98) is not thought to be associated with the defective phenotype of the mutant. The longer repetition (bp 281–340) duplicates a region spanning the *E1A* promoter-proximal ("−200") enhancer core element identified in Ad5 by Hearing and Shenk (13); this enhancer core DNA sequence is identical in the Ad3

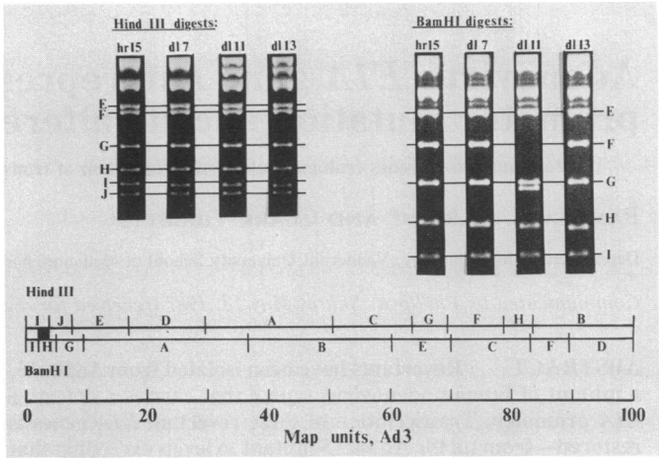

FIG. 2. DNA restriction enzyme analysis of Ad3hr15 revertants. Restriction enzyme digests of Ad3hr15, and the revertants dl 7, dl 11, and dl 13 are shown using *Hin*dIII and *Bam*HI at the left and right, respectively. Deletions occur in the revertant DNAs in the interval from bp 840 (*Bam*HI) to bp 1476 (*Hin*dIII) shaded in the restriction enzyme map below the digests (1 map unit = 350 bp). The *Bam*HI–F fragment of revertant dl 11 also bears a deletion that probably affects the nonessential early gene *E3*.

and Ad5 genomes. Marker rescue experiments confirmed that the defective lesion of Ad3hr15 is located left of the *Pvu* II restriction site, bp 465 (8).

The Ad3hr15 mutant is remarkably defective and can only be propagated in the 293 cell line (14–16), which provides *E1A* gene products of the heterologous Ad5. Although A549 cells are normally permissive for growth of Ad3 or Ad5, they do not support growth of the mutant Ad3hr15 virus. This provided an opportunity to select for phenotypic revertants of Ad3hr15 by high-multiplicity (1250 particles per cell) infection of A549 cells. Lysates of such infected cell cultures were subjected to plaque titration on A549 cells for isolation of candidate revertant viruses. DNA purified from revertant virion preparations was characterized by DNA restriction analysis (Fig. 2). There was no apparent change in the size of the left *Bam*HI fragments in the revertant DNA digests, compared with Ad3hr15. However, each of the phenotypic revertants incurred a small deletion of DNA in the region between the left *Bam*HI and *Hin*dIII restriction sites. These revertant-specific *E1A* deletions varied from ≈20 to ≈100 bp in length. Other restriction fragment-size changes were noted among the 45 revertants that were screened, but none of these was consistently associated with the reverted growth phenotype. Thus, reversion of the defective growth phenotype of Ad3hr15 resulted from second-site deletions in the *E1A* gene, rather than by alteration of the duplicate enhancer structure of the Ad3hr15-type *E1A* promoter. The infectivity of rever-

Table 1. Infectivity of Ad3, mutant Ad3hr15, and revertants

Virus	Particles, pfu	
	A549 Cell line	293 Cell line
Wild-type Ad3	11	1000
Defective Ad3hr15	1.3×10^7	300
Revertant Ad3hr15-dl 7	150	290
Revertant Ad3hr15-dl 13	690	650

Purified virions were serially diluted in phosphate-buffered saline (PBS-A) and 2% calf serum for plaque assay. Procedure details used for virus growth, purification of virions, and assay of infectivity have been described in reports from this laboratory (8–10). Plaques of Ad3hr15 in A549 cells are abnormal in size and do not appear proportionally to dilution, as earlier reported (8); the mutant is thus more defective in A549 cells than suggested by the above data.

tants such as dl 7 and dl 13 was several orders of magnitude greater in A549 cells than the infectivity of mutant Ad3hr15 (Table 1). The infectivity of these revertants in 293 cells was similar to that of of the mutant Ad3hr15, ≈2 to 3 times higher than for wild-type Ad3 in A549 cells.

Physical Mapping of DNA from Revertant Viruses. Revertants dl 7 and dl 13 (Fig. 2) closely resemble the overall genotype of the Ad3hr15 mutant from which they originated, except for their *E1A* gene deletions. Partial digestion restriction analysis (11, 17) of revertant viral DNA preparations further delimited the location of the deletions to the interval between the *Pvu* II (bp 908) and *Alu* I (bp 1111) restriction sites in the Ad3 *E1A* gene (results not shown). Maxam–Gilbert DNA sequence analysis (12) defined these revertant deletions, aligned with the sequence of Ad3 DNA (a) from bp 950 to bp 1030 below. [Translated amino acids in one-letter code are shown on the line between (a) and (b)]:

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   950                                                                    1030
(a) GATGATGAAGATGGGGAAACTGAGCAGTCCATCCATACCGCAGTAAATGAGGGAGTAAAAGCTGCCAGCGATGTTTTTAAG
      D  D  E  D  G  E  T  E  Q  S  I  H  T  A  V  N  E  G  V  K  A  A  S  D  V  F  K
(b) GATGATGAAGATGGGGAAACTGAG------------------------GGAGTAAAAGCTGCCAGCGATGTTTTTAAG
(c) GATGAT------------------------------------------------------------TTTAAG
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Revertant dl 7 (b) deleted 27 bp, 974–1000 inclusive (Ad3 nucleotide numbers), and revertant dl 13 (c) deleted 69 bp, 956–1024 inclusive. These overlapping deletions thus maintained the translational reading frame, removing 9 or 23 codons from a region that is common to the 5′ exons of 12S and 13S Ad3 *E1A* mRNA species.

Restored Capacity for *E1A* Gene Expression by Revertants. The results of RNA blot analysis (Fig. 3) demonstrate *E1A* gene expression by wild-type Ad5 and Ad3 and the transcriptional defect of the Ad3hr15 promoter. Wild-type Ad3 exdo wild-type Ad3-infected 293 cells (data not shown). Ad3hr15 also shows transcription of its *E1A* gene in cells coinfected with Ad5. In these cells, however, the appearance of the *E1A* transcripts from the mutant is so late in the coinfection that the splicing appears specific for generation of the late-9S form of the mRNA. It is not known whether these late-mRNA forms generated by Ad3hr15 (or the wild-type Ad3 or Ad5) would be effectively translated, or whether their putative translation products are capable of any *E1A*-like functions. Analysis of Ad5 *E1A* RNA from these coinfected cells (data not shown) gave results similar to those of Fig. 3*A*.

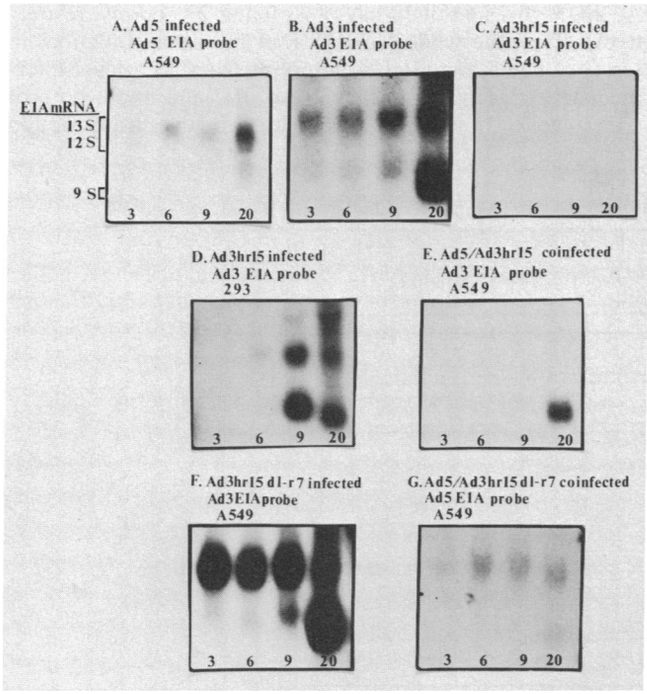

FIG. 3. *E1A* gene expression by Ad5 and the Ad3 wild-type, mutant, and revertant viruses. Text indicates the virus(es) used to infect cells (A549 or 293) and the type (Ad3 or Ad5) of *E1A*-specific DNA probe hybridized with blotted RNA. RNA was isolated from cells at 3, 6, 9, and 20 hr after infection, as indicated. ITR, inverted terminal repetition.

A549 cells infected by the revertant dl 7 yielded even higher levels of its *E1A* mRNA species than observed for wild-type Ad3 (Fig. 3 *F* and *B*). In another experiment A549 cells were coinfected with Ad5 and the dl 7 revertant. RNA from the coinfected cells was separately analyzed using Ad5- or Ad3-specific *E1A* probe DNA. The levels of *E1A* mRNA in the revertant (detected with Ad3 probe) at different times after the coinfection were similar to the mRNA in cells infected by dl 7 virus alone (data not shown). Ad5-*E1A* mRNA was also detected in these coinfected A549 cells (Fig. 3*G*). Although these Ad5 transcripts were at somewhat lower levels than in cells infected by Ad5 alone, there was no apparent delay in their appearance after infection. Coinfection experiments with Ad5 and Ad3hr15–dl 13 led to similar results as with dl 7 and Ad5 (data not shown). It has been previously reported (1) that wild-type Ad3 virus completely represses expression of Ad5 *E1A* under these coinfection conditions. It thus appears that the elevated levels of revertant of dl 7-type *E1A* products, inferred from the abundant mRNA, are less capable of repressing transcription from the Ad5 *E1A* promoter than are wild-type Ad3 *E1A* products.

DISCUSSION

Very early after infection of A549 cells by Ad3hr15 mutant virus the mutant *E1A* promoter is presumed to function effectively in the absence of any Ad3 *E1A* products. The wild-type *E1A* products that subsequently result from this initial phase of *E1A* gene expression by Ad3hr15 then return to the nucleus and block further *E1A* gene transcription. The infection by Ad3hr15 apparently aborts because of insufficient expression of *E1A* and other *E1A*-dependent genes. Although the initial phase of *E1A* gene expression is probably

presses its *E1A* gene at earlier times after infection and with much greater levels of mRNA in infected A549 cells than does Ad5 (Fig. 3*A* and *B* and ref. 1). *E1A* transcription by the mutant Ad3hr15 is almost completely blocked in A549 cells (Fig. 3*C*). A barely discernible amount of the late-specific 9S mRNA species appears at 20 hr after Ad3hr15 infection of these cells. This minute signal is perhaps associated with events that eventually lead to the appearance of nonproductive microplaques after such high-multiplicity infections by the mutant virus.

The blocked transcription of the Ad3hr15 *E1A* gene appears relaxed in cells that concomitantly express the Ad5 *E1A* gene (Fig. 3 *D* and *E*). The Ad3hr15-infected 293 cells accumulated abundant Ad3-type *E1A* transcripts after a delay of several hours, similar to that for Ad3-infected 293 cells reported previously (1, 8). By 9 hr after infection, however, the Ad3hr15-infected 293 cells show more *E1A* mRNA than

the same in revertant virus-infected A549 cells, subsequent effects of the *E1A* gene products upon transcription (from the Ad3hr15-type *E1A* promoter of the revertant) are dramatically different. The revertants of Ad3hr15 overexpress their mutated *E1A* genes in comparison with the normally intense expression of *E1A* by wild-type Ad3 in A549 cells.

We attribute these high levels of *E1A* gene expression by the revertants of Ad3hr15 to diminished autorepression of the *E1A* gene. This conclusion comes from these observations: (*i*) transcription block from the Ad3hr15 promoter was relaxed by deletions within the 5′ coding region of the Ad3 *E1A* gene, and (*ii*) revertant *E1A* gene products have diminished capacity to repress transcription of the heterologous Ad5 *E1A* gene in coinfected cells. The Ad3hr15 *E1A* promoter has a reiterated enhancer element, compared with the wild-type Ad3 *E1A* promoter, which may explain the enhanced sensitivity of the mutant to *E1A* autorepression. However, this sensitive *E1A* promoter of Ad3hr15 remains responsive to autoactivation as seen when the revertant E1A proteins shift the net balance of Ad3hr15 *E1A* gene autoregulation from the repressed to the activated state.

The *E1A* promoter of mutant Ad3hr15 and the coding sequences of the *E1A* genes of revertants dl 7 and dl 13 have been cloned by substitution of the corresponding DNA sequences in the Ad3pE1A and Ad3pE1ACAT plasmids described previously (1). Transient gene expression analysis in transfected and cotransfected HeLa cells have shown that the duplicated enhancer region of the mutant is not a cis-acting lesion because the promoter functions effectively in the absence of *E1A* gene products. The mutant *E1A* promoter responds to both positive and negative regulation by *E1A* gene products in trans. The deleted forms of the Ad3hr15 *E1A* genes of the revertant are diminished in capacity to repress gene expression from enhancer-associated promoters (S. Jones and C.T., unpublished work).

Deletions in the *E1A*-coding region of the revertants dl 7 and dl 13 suggest a functional domain within the Ad3 E1A protein(s) that is required for *E1A* gene autorepression activity and, perhaps, also for repression of transcription units that are associated with other enhancer elements (4–7). Fig. 4 illustrates the region of the Ad3hr15 revertant deletions aligned with the corresponding amino acid sequences of different adenoviruses (18, 19). The region shows evolutionary variation in the length of coding sequences that separate more highly conserved regions. Perhaps the capacity for *E1A* gene autorepression of the different adenoviruses is reflected in these divergent DNA and amino acid sequences. We predict that a longer gap in the sequence alignment would reflect lower capacity for autorepression, as in the case of wild-type Ad3 compared with the dl 7 and dl 13 revertants. The 12S mRNA splice-donor sites of the different adenoviruses have been located in different positions relative to the alignment of their homologous DNA sequences (18, 19). A consequence of these different splicing sites is that the variable-length domain is common to the 12S and 13S mRNA translation products of A and B subgroup adenoviruses (Ad12 and Ad3), but the region is uniquely specified by the 13S mRNA translation products of the C subgroup Ad5 and simian adenovirus SA7.

Recent studies of *E1A* deletion mutants and missense mutations of Ad5 have identified a domain that is critical for Ad5 *E1A*-related functions of oncogenic cell transformation and transcriptional repression (20–23). These Ad5 *E1A* mutations are located in the highly conserved DNA sequences immediately upstream from the functional domain that is associated with the *E1A* autorepression function. Perhaps these two adjacent regions interact to determine the specific activity of E1A proteins in transcriptional regulation of different cellular genes involved in transformation. We note with interest that our Ad3hr15 revertant dl 13 deletes a highly conserved glutamate codon (E), which is the locus of a missense mutation (at nucleotide 961 of Ad5, ref. 23) that impairs the transforming potential of the Ad5 *E1A* gene.

Evolution has led to differences between Ad3 and Ad5 in the autorepression domain of the *E1A* gene that can explain the capacity of Ad3hr15 to replicate and express its *E1A* gene when in the presence of Ad5 *E1A* gene products (infection of 293 cells, Fig. 3*D*, or coinfection of A549 cells, Fig. 3*E*). Fig. 4 shows that Ad5 *E1A* has 16 fewer codons than does wild-type Ad3 *E1A* in this interval, whereas the revertants of Ad3hr15, dl 7 and dl 13 have lost 9 and 23 codons, respectively. Thus the wild-type Ad5 *E1A* gene may function, in terms of heterologous *E1A* autorepression, at a level intermediate between the *E1A* genes of dl 7 and dl 13, both of which show no net restriction on viral growth or transcription from their Ad3hr15-type *E1A* promoters.

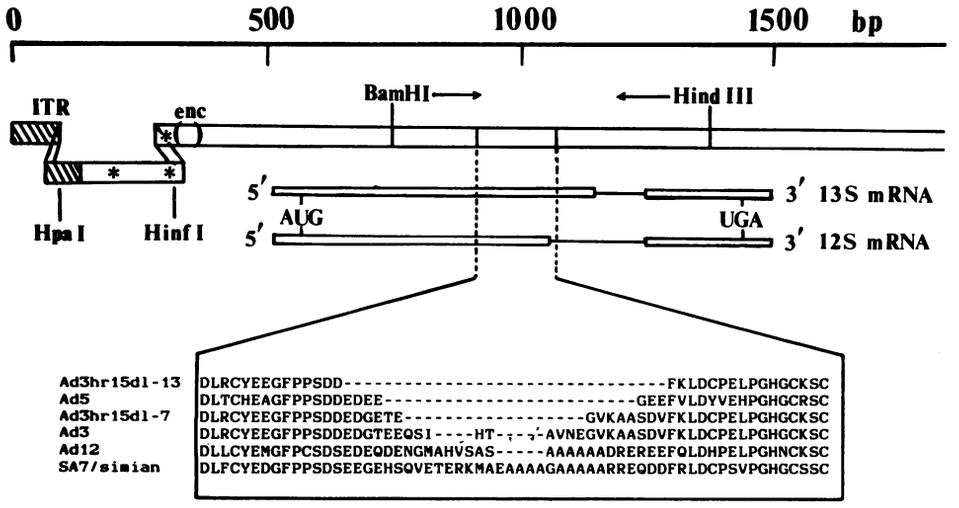

FIG. 4. *E1A* gene functional domain for autorepression. Maps of the *E1A* region at the left end of mutant Ad3hr15 DNA and the Ad3 *E1A* mRNA species are as described in the legend for Fig. 1. Revertants of Ad3hr15 have deletions between the indicated *Bam*HI and *Hin*dIII restriction sites (Fig. 2). Beneath maps partial amino acid sequences (single-letter code) for representatives of different adenovirus subgroups are shown: Ad5, human C; Ad3, human B; Ad12, human A; and simian adenovirus SA7. Alignment of sequences is from Kimelman *et al.* (18). Revertant viruses dl 7 and dl 13 were inserted into the list by alignment of their amino acid sequences and the length of gap apparent from comparison with conserved domains at the ends of the region. ITR, inverted terminal repetition.

Trans-activation of viral and cellular transcription units by the adenovirus *E1A* gene has been specifically associated with a physically separate functional domain, the 13S mRNA-specific portion (46 codons) of the Ad5 *E1A* gene (20–26). This positive regulatory domain corresponds to Ad3 DNA sequences that are located downstream from the region that we report here to be associated with Ad3 *E1A* autorepression.

The cell transformation frequency of each of the adenovirus subgroups is similar; however, the tumorigenicity of adenoviruses or adenovirus-transformed cells varies widely (18, 27, 28). Variations in tumorigenicity have been attributed to the differential effects of adenovirus *E1A* gene expression upon the levels of class I major histocompatibility complex (MHC) cell surface antigens (29–33) in the transformed cells. This property of the *E1A* gene is determined by the sequences in its 5′ exon, as revealed in studies of adenoviruses having chimeric Ad5–Ad12 *E1A* genes (34). The length of the DNA sequence in the *E1A*-autorepression functional domain is correlated with the gradient of increasing adenoviral tumorigenicity; Ad5 < Ad3 < Ad12 < SA7. Because the region of the Ad5 *E1A* transformation and repression mutants is strongly conserved among the different adenovirus subgroups, as mentioned above, we propose that the adjacent functional domain associated with enhancer-targeted (auto) repression may be the source of the variation of tumorigenic potential among the adenoviruses. Original or different DNA sequences at the deletion junctions of the *E1A* genes of revertants dl 7 or dl 13 can be reinserted by site-directed mutagenesis *in vitro*; this technique would help determine how the oncogenic effects and enhancer-targeted repression activity of *E1A* gene products are modulated by this gene region. Such experiments should distinguish between the importance of specific coding for DNA sequences versus precise physical separation and alignment of the adjacent conserved domains in the multifunctional E1A proteins.

We appreciate careful reading of early drafts of the manuscript by Stephen Jones. Work by Robert Hall of this laboratory has produced the DNA sequence of the entire Ad3 *E1A* gene that is referred to throughout this paper. Thanks are also due to Stanley Cohen and Tom Shenk for their interest and comments. This work was supported by U.S. Public Health Service Grant CA-34126 from the National Cancer Institute; P.L.L. was supported in part by the Cellular-Molecular Biology Graduate Training Program Grant GM-07319 from the Institute of General Medical Sciences.

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