JOURNAL OF VIROLOGY, July 1990, p. 3427–3436
0022-538X/90/073427-10$02.00/0

Vol. 64, No. 7

The Efficiency of Adenovirus Transformation of Rodent Cells Is Inversely Related to the Rate of Viral E1A Gene Expression

GUY R. ADAMI AND LEE E. BABISS*

Laboratory of Molecular Cell Biology, The Rockefeller University, Box 279, 1230 New York Avenue, New York, New York 10021-3699

Received 6 February 1990/Accepted 16 April 1990

While the products of the type 5 adenovirus E1A and E1B genes can initiate pathways leading to a transformed rodent cell, little is known about how the rate of viral early gene expression influences the efficiency of this process. An adenovirus mutant [E1a(r) virus] that expresses its viral E1A and E1B genes at as much as a 100-fold-reduced rate relative to wild-type virus in infected CREF or HeLa cells transforms CREF cells at an 8-fold-higher efficiency than wild-type virus. Additional studies show that the reduction in viral E1A gene expression is solely responsible for this transformation phenotype, and at this low rate of viral E1A gene expression both E1A gene products must be expressed. Unlike previously characterized viruses which transform CREF cells at frequencies greater than wild-type virus, the foci obtained following E1a(r) virus infection were indistinguishable from those arising from wild-type virus by several criteria (morphological characteristics and anchorage-independent growth). Surprisingly, an analysis of viral early gene expression from a panel of wild-type- and E1a(r) virus-transformed CREF cell lines showed similar average rates of both viral E1A and E1B gene expression. By using an adenovirus-transformed cell line that is cold-sensitive for maintenance of the transformed cell phenotype, we show that both wild-type and the E1a(r) viruses can transform these cells at equally high efficiencies at the nonpermissive temperature of 32°C. Our findings suggest that the process leading to a fully transformed cell involves multiple stages, with an early stage being facilitated by a reduced rate of viral E1A gene expression.

Changes in the temporal regulation of cellular gene expression and DNA replication incurred by viral oncogene expression can be manifested by the generation of an immortalized or transformed cell phenotype. For adenovirus type 5 (Ad5), viral E1A and E1B gene expression in a susceptible rodent cell type can lead to the initiation and maintenance of a morphologically transformed cell (for a review, see reference 17). Transcription from the viral E1A gene leads to the production of two differentially spliced mRNAs which encode proteins of 289R (amino acid residues) and 243R (8, 11, 29, 39). These viral E1A gene products have been shown to alter many biological functions in virus-infected rodent and human cells, including transcriptional transactivation (7, 16, 27, 37, 48, 50) and repression of both viral and cellular genes (9, 23, 46, 51), the induction of cellular DNA synthesis in quiescent cells (31, 41), the immortalization of primary rodent cells (26, 44), and complete morphological transformation of primary or continuous cultures of rodent cells when coexpressed with the viral E1B gene or certain viral oncogenes such as *ras* (19, 28, 33, 35, 36, 55, 57).

The viral E1B gene-encoded proteins have been shown to play a role in the shutoff of host cell protein synthesis and the efficient nuclear-cytoplasmic transport of late viral mRNAs in the infected cell (3, 4, 40), the association and alteration of the organization of intermediate filaments and the nuclear lamina (52, 53), cooperation with the E1A gene products to initiate cellular pathways leading to a transformed cell (for a review, see reference 17), and maintenance of a morphologically complete transformed cell phenotype (2, 6, 26, 53). Both the viral E1A and E1B proteins have been shown to physically associate with several cellular proteins in the infected cell, but the consequences of these associations as they relate to the host range of virus-induced transformation is unclear (20, 45, 54–56).

Like most DNA tumor viruses, the efficiency of focus formation following adenovirus infection of rodent fibroblast cells is poor (at most, 1 in 300 infected cells) (5). Explanations for this low frequency of transformation induction could include (i) the presence of only a limited number of cells in the infected cell population that have the potential to become transformed or (ii) the presence of a uniform population of cells, with transformation depending on the occurrence of several random events. Transformation rates could be affected by variations in such factors as initial rates of viral gene expression, location and frequency of viral DNA integrations, and levels in viral gene expression following viral DNA integration.

For many viral oncogenes, a correlation between the rate of oncogene expression and the frequency of cellular transformation has been established. Usually, overexpressing the viral oncogene product in a susceptible cell (either in a plasmid vector or following virus infection) leads to a higher frequency of cellular transformation and established transformed cell lines that display a more progressed transformed cell phenotype (10, 32, 38). In fact, Senear and Lewis (47) have shown that when the Ad2 E1A gene is overexpressed on a plasmid vector in the absence of the viral E1B gene in mouse NIH 3T3 cells, stable transformants that are phenotypically indistinguishable from foci which coexpress the viral E1A and E1B genes are obtained. Therefore, the level of viral oncogene expression can influence the requirement for a cooperating viral gene product. Finally, overexpressing the polyomavirus middle T antigen in rodent F111 cells (a continuous culture of rodent fibroblast cells), leads to a decrease in the efficiency of focus formation (42).

In this paper, we explore how the rate of viral E1A and E1B gene expression influences the efficiency of CREF cell

* Corresponding author.

transformation. We observed previously that the rate of transcription from the viral E1A gene in wild-type-virus-infected CREF cells was reduced by 5- to 10-fold when compared with a similar wild-type virus infection of human HeLa cells (24, 25). Additional studies showed that an adenovirus mutant (*dl*309-6) lacking DNA sequences between −393 and −304 base pairs relative to the E1A gene cap site (22) expressed E1A mRNA levels that were 5- to 10-fold greater than those of the wild-type virus upon infection of CREF cells (25). A virus [E1a(r)] containing an E1A gene reversed in orientation relative to the E1B gene, expressed 20- to 100-fold less E1A and E1B mRNAs than wild-type virus following CREF and HeLa cell infection. However, when these same viruses were used to infect CREF cells for extended time periods [24 or 88 h postinfection for the *dl*309-6 or E1a(r) viruses, respectively], E1A mRNA levels approached those observed for wild-type virus.

By using these viruses, our studies show that reducing the rate of viral E1A gene expression leads to an eightfold increase in foci numbers relative to wild-type virus, and the foci are morphologically indistinguishable from those arising from wild-type virus infection. In contrast, overexpressing the viral E1A gene leads to a modest two-fold decrease in foci numbers, when compared with wild-type virus. Additional virus complementation studies show that the reduction in E1A and not E1B gene expression is responsible for the E1a(r) virus transforming phenotype and that viruses which poorly express E1A mRNAs must express both E1A gene products to be transformation competent. These and additional observations suggest that a reduced rate of viral E1A gene expression early after CREF cell infection has a profound effect on transformation frequency.

MATERIALS AND METHODS

Cells and viruses. Monolayer cultures of human HeLa and 293 (a human embryo kidney cell line that constitutively expresses the type 5 adenovirus E1A and E1B gene products [18]) cells were grown in Dulbecco modified Eagle medium supplemented with 10% defined calf serum. Monolayer cultures of CREF (13) and the Ad5hr1-transformed CREF cell line A2 (5) were maintained in the same medium supplemented with 5% fetal bovine serum (D-5 medium). Viral stocks from the viruses shown in Fig. 1 were obtained as described earlier by using the 293 cell line (3, 25). The Ad5hr1 virus was obtained from J. Williams and contains a point mutation that leads to the production of a truncated viral E1A 13S gene product (21, 43). The *dl*309-6 virus was obtained from P. Hearing and contains a deletion that removes a protein-binding domain that represses E1A enhancer activity in CREF cells (22, 25). The viral E1A mutants *pm*975 (expresses no E1A 12S gene product) and *dl*1500 (expresses no E1A 13S gene product) were obtained from A. Berk (33, 34), and H5*dl*110 (no E1B 495R protein expression) and H5*dl*112 (no E1B 175R protein expression) have been previously described (3). The α3-3 virus contains the mouse immunoglobulin heavy-chain promoter and enhancer localized between viral sequences 454 and 1109 (14). This virus retains its E1A enhancer element but lacks the E1A cap site and much of the protein-encoding sequences. The E1B gene is in its wild-type location on the viral genome and is expressed at wild-type rates following CREF or HeLa cell infection (data not shown).

Construction of the E1a(r) virus. The Ad5 *Xho*I C fragment (nucleotides 0 to 5788) cloned into the plasmid vector pMK2004 has been described previously (5). The EAB8 plasmid was derived from the pMK plasmid and contains viral nucleotides 0 (*Bam*HI) to 4623 (*Apa*I), blunt ended and inserted into the unique *Sma*I site in the pUC8 plasmid vector. The E1a− plasmid contains the E1A gene without its 3′ end, cloned into the plasmid vector Bluescript plus. It was constructed by digesting the pMK plasmid with *Sac*II (nucleotide 357), blunt ending this site, digesting with HpaI (nucleotide 1574), and blunt-end-ligating this fragment into the unique *Hin*cII site in Bluescript. The *Hin*cII site was retained only at the 3′ end of the E1A gene insert. To restore the 3′ end of the E1A gene, a duplex oligonucleotide was synthesized containing nucleotides 1601 to 1663 with a *Sac*II sticky end at nucleotide 1663. The E1a− plasmid was digested with *Sac*II and *Hin*cII, and the oligonucleotide (nucleotides 1601 to 1663) containing a unique SacII site was inserted. This is the E1a+ plasmid, which contains the E1A gene promoter, coding region, and polyadenylation signals. As 23 bases have been deleted upstream of the polyadenylation signal, this message is able to be differentiated from wild-type E1a mRNA. The EAB8 plasmid was digested with SacII (nucleotide 357) and *Kpn*I (vector) and was ligated to a *Kpn*I (vector)-to-*Sac*II (nucleotide 1663) fragment derived from the E1a+ plasmid. The result was a plasmid, E1a(r), containing the E1a gene and its promoter, both in reverse, fused to the 352 bases at the left end of the viral genome. This plasmid was digested with *Bam*HI (nucleotide 0) and *Xho*I (blunt ended) and cloned into the *Bam*HI and *Hpa*I sites of the pMK plasmid. Thus, it contained the left end of the viral genome, extending from nucleotides 0 to 5788, with the sequences between nucleotides 357 and 1663 reversed in orientation. The inverted E1A gene was fused at nucleotides 357 and 1574 of the wild-type genome, so approximately 100 bases were duplicated. These sequences were introduced into a complete viral genome by in vivo overlap recombination in human 293 cells as previously described (3), followed by two rounds of plaque purification on 293 cells.

Transformation of CREF and A2 cells and anchorage-independent growth. Transformation assays were performed as described previously (24). Prior to virus infection, the A2 cell line was maintained at 32°C for 4 weeks, at which time all the cells in culture displayed a fibroblastic CREF cell appearance. The effect of the various viruses on cell survival was determined by plating mock-infected or virus-infected cells at 200 and 400 cells per 60-mm (diameter) plate and quantitating the viable cells per dish following formaldehyde fixation and Giemsa staining. Cell survival was calculated by dividing colonies on the infected plates by the number on mock-infected controls. Once well-defined foci could be discerned and quantitated, several foci were isolated by using cloning cylinders, recloned following plating, and expanded into established cell lines that were maintained in D-5 medium. Soft-agar cloning assays were performed as previously described (5).

Assays for gene expression. Cytoplasmic RNA was isolated from virus-infected CREF and HeLa cell cultures, and virus-transformed CREF cell lines as previously described (1, 24). The production of stable mRNAs from the viral E1A and E1B genes was scored by using ^{32}P-labeled SP6-generated antisense E1A and E1B probes. Following hybridization and RNase T2 digestion, the products were sized on denaturing polyacrylamide gels. The RNase T2 protection assay for these viral mRNAs has been previously described (24). The sizes of the protected RNAs are shown in Fig. 1.

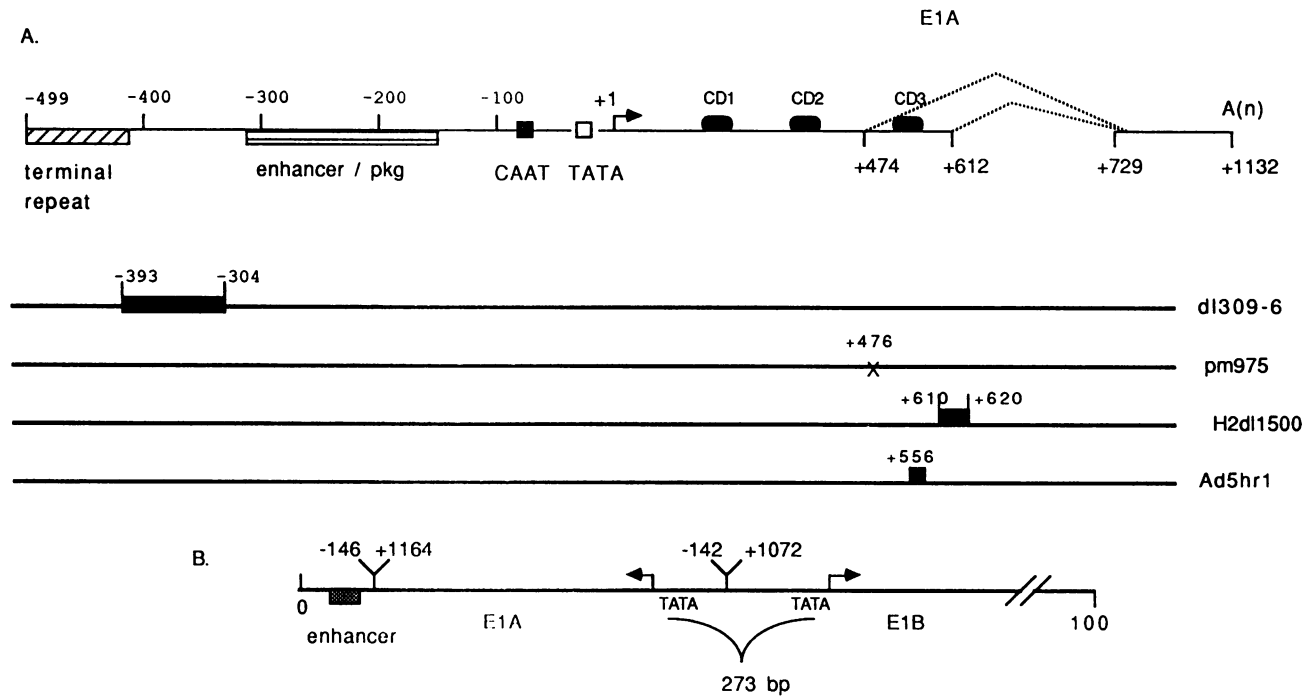

FIG. 1. Schematic representation of the adenovirus E1A gene transcription unit and promoter-proximal and enhancer regions. (A) Left end of the adenovirus genome, with relative nucleotide locations of the viral DNA regions that contribute to E1A promoter function (enhancer-packaging sequence [▤], CAAT box [▨], and TATA box [□]) and viral DNA replication (terminal repeat [▨]) relative to the E1A gene cap site. The nucleotide locations of the various RNA splice donor and acceptor sites, and polyA addition sites are shown for the E1A 13S and 12S mRNAs. Three regions of conserved amino acid coding domains for various viral serotypes are shown (CD1 to CD3) relative to their locations on the viral mRNAs (35). As shown, CD3 is unique to the E1A 13S mRNA. The viral genomes shown contain various deletions that alter viral E1A gene expression, and their isolation and characterization have been described previously (21, 22, 33, 34). DNA deletions (■) and a single base pair deletion (X) are also shown. The nucleotide locations of these deleted viral DNA sequences are shown in base pairs relative to the E1A gene cap site. (B) Diagram of the E1a(r) virus genome, which shows how the E1A gene was reoriented so that it is now transcribed in a leftward direction. All nucleotide positions are again shown relative to the E1A gene cap site.

RESULTS

Construction of the E1a(r) virus. In an attempt to study the *cis* effect of adenovirus E1A gene transcription on the rate of E1B gene transcription, a virus was constructed that contained its wild-type E1A gene in a reversed orientation relative to the E1B gene promoter [Fig. 1B, E1a(r) virus]. Therefore, no overlapping E1A gene transcription occurs across the E1B gene cap site, since E1A transcription now proceeds from right to left on the viral genome. Like wild-type virus, the E1A 289R and 243R proteins should be faithfully expressed from this altered viral genome. The functional activity of the 289R protein expressed by the E1a(r) virus was confirmed by coinfecting CREF or HeLa cells with the E1a(r) virus and a virus containing the E1A 289R protein-inducible human β-globin promoter (31). The E1a(r) virus *trans*-activated β-globin expression at rates compatible with the E1A mRNA levels observed (data not shown). As will be shown, the functionality of the 243R protein was suggested by the ability of the E1a(r) virus-transformed cell line to proliferate in soft agar.

E1a(r) virus expresses low levels of E1A and E1B mRNAs following CREF or HeLa cell infection. Viral E1A and E1B gene expression was examined in CREF and HeLa cells infected with 20 PFU per cell of wild-type *In*340 virus, *dl*309-6 virus, or E1a(r) virus. At the times shown at the top of each panel in Fig. 2, total cytoplasmic RNA was isolated and analyzed for the abundance of the viral E1A 13S and 12S mRNAs (HeLa cells [Fig. 2B]) or E1A and E1B mRNAs (CREF cells [Fig. 2A and C]). In both cell types, E1A mRNA accumulation from the E1a(r) virus was reduced by 20- to 100-fold (by densitometric quantitation) relative to the level observed in *In*340 virus-infected cells at either time point (Fig. 2); the late time points were overexposed to allow us to score E1a(r) virus gene expression. The E1A mRNAs

TABLE 1. Reducing the rate of viral E1A gene expression increases the frequency of CREF cell transformation[a]

Virus	MOI	Genotype[b]	E1A expression (fold)[c]	Mean foci/dish[d]		% Clonal survival[e]
				Expt 1	Expt 2	
*In*340	10	WT	1	19	22	93
*dl*309-6	10	ΔφAP3	5	8.5	12.5	93
E1a(r)	10	Reversed E1A	0.01	134	167	95
Mock				0	0	92

[a] CREF cells were infected at the multiplicity of infection (MOI) shown, and following adsorption, cells were suspended and replated at 10^5 cells per 60-mm (diameter) plate for 5 weeks at 37°C.

[b] The orientation of the E1A gene on the viral genome and the E1A mRNAs which are expressed is shown. WT, Wild type.

[c] The rate of E1A gene expression following an 8-h infection of CREF cells at the multiplicity of infection shown for each virus is shown relative to the level observed for *In*340.

[d] Mean number of transformed cell colonies; each value represents the quantitation of 6 to 10 plates.

[e] Percentage of surviving cells following virus infection, as determined by plating 50, 100, or 200 cells per 100-mm (diameter) plate, allowing well-defined colonies to develop in 10 to 12 days, fixing and staining the cells, and counting the colonies.

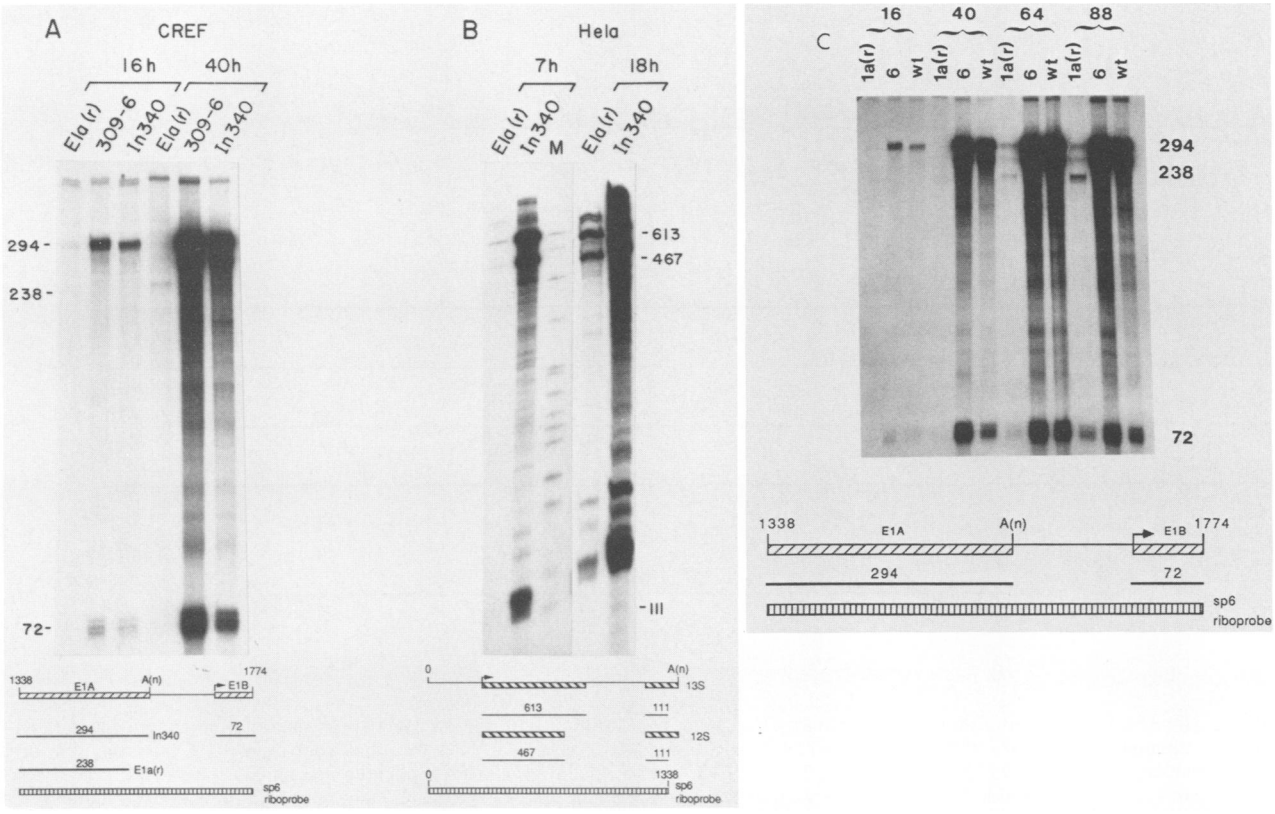

FIG. 2. The E1a(r) virus expresses low levels of its E1A and E1B gene mRNAs upon CREF and HeLa cell infection. CREF (A) and HeLa (B) cells were infected with 20 PFU of the viruses shown per cell for the times indicated. Total cytoplasmic RNA was isolated and scored for the presence of the E1A and E1B mRNAs (A) or E1A mRNAs (B) by RNase T2 protection assays. Total cytoplasmic RNA (15 μg) was hybridized to [^{32}P]UTP-labeled antisense RNA probes (7.5×10^5 cpm) for each of the probes shown in diagrams at the bottom, and the RNase T2 digestion products were analyzed on 5% denaturing acrylamide gels. The lengths of the protected RNAs are shown at the bottom. The 3′ ends of the E1A mRNAs could be distinguished between *In*340 and E1a(r) viruses. Monolayers of CREF cells were infected with 20 PFU per cell with the viruses shown for the times indicated (C). Total cytoplasmic RNA was isolated and scored for the presence of E1A and E1B mRNAs, as described above for panel A. With the later time points for E1a(r) virus, it is likely that the bands at nucleotide 294 correspond to an RNase T2 digestion artifact.

expressed from the E1a(r) virus genome could be distinguished at their 3′ ends in the CREF cell assay from wild-type virus due to a 23-base-pair deletion in the noncoding region (Fig. 2A, diagram). The faint band of 294 nucleotides seen at 16 h after infection of CREF cells by the E1a(r) virus was not reproducibly observed and represents an RNase T2 digestion artifact (note the absence of this band in Fig. 4, lane 7). The same reduction in E1B mRNA levels in E1a(r) virus-infected CREF and HeLa cells was observed (Fig. 2; data not shown). Between 2 and 4 days following CREF cell infection by wild-type *In*340 virus, E1A and E1B steady-state mRNA levels plateaued (Fig. 2C). In contrast, E1A mRNA levels following a similar time course of CREF cell infection with the E1a(r) virus were low but continued to increase (Fig. 2C). That transcriptional control and not mRNA turnover was contributing to the E1a(r) virus phenotype was confirmed by measuring rates (by nuclear runon experiments) of viral E1A and E1B gene expression in *In*340 and E1a(r) virus-infected CREF cells (data not shown).

The *dl*309-6 virus contains a deletion of viral DNA sequences directly upstream of the E1A enhancer domain that releases the block on E1A gene enhancer-dependent expression in virus-infected CREF cells (Fig. 1) (25). Following CREF cell infection by this virus (20 PFU per cell for 16 h), a modest increase (about twofold) in the steady-state accumulation of the viral E1A and E1B mRNAs was observed (Fig. 2A). However, at earlier stages of the *dl*309-6 virus infection of CREF cells (7 and 12 h postinfection), E1A expression was 5- to 10-fold greater than that observed from *In*340 virus (25).

Reducing the rate of viral E1A and E1B gene expression increases the frequency of CREF cell transformation. To correlate the rate of viral E1A and E1B gene expression and the efficiency of cellular transformation, CREF cells were infected with the three viruses that expressed their E1A genes at different rates [*In*340 virus (wild type), *dl*309-6 virus (5- to 10-fold increase in E1A), or E1a(r) virus (20- to 100-fold decrease in E1A) at a multiplicity of infection of 10 PFU per cell. Since E1A expression can be cytotoxic (24, 33), clonal survival assays were done following each virus infection, and the results were found to be similar for all the viruses (Tables 1 and 3). Following a 5-week incubation period at 37°C for two independent experiments, the cells were fixed and stained and the foci per dish were quantitated. When compared with *In*340 virus, *dl*309-6 virus produced roughly one-half as many foci that displayed a transformed morphological phenotype that was indistinguishable from wild-type virus (Table 1). This suggests the possibility that an increase in the rate of viral E1A gene expression does

TABLE 2. Cloning efficiency in soft agar of uninfected, E1a(r)-transformed, *In*340-transformed, and Ad5hr1-transformed CREF cells grown at different temperatures[a]

Cell line	% (Mean ± SD) cloning efficiency	
	37°C	32°C
CREF	<0.001	<0.001
A2—Ad5hr1	72 ± 2	15 ± 1
*In*340—clone 1a	69 ± 1	47 ± 1
*In*340—clone 5	81 ± 2	67 ± 1
*In*340—clone 7	49 ± 4	35 ± 6
E1a(r)—clone 1	68 ± 3	50 ± 4
E1a(r)—clone 4	75 ± 4	54 ± 6
E1a(r)—clone 8	79 ± 5	ND

[a] Approximately 10^3 or 10^5 cells were suspended in low-Ca^{2+} medium containing 0.4% Noble agar and seeded at 32 or 37°C on 0.8% agar base layers prepared in the same medium. Plates were fed once a week with 3 ml of 0.4% agar in low-Ca^{2+} medium, and microscopic colonies were counted after 2 to 3 weeks (37°C treatment) and after 6 weeks (32°C treatment) for cultures. Each value is the mean ± standard deviation (SD) of three to five plates. The A2-Ad5hr1 colonies counted at 32°C were consistently four to five times smaller than the colonies counted at 37°C for all the cell lines and the colonies grown at 32°C from the E1a(r)- and *In*340-transformed cell lines, as previously observed (5). ND, Not determined.

not dramatically change the transforming phenotype of this virus.

In contrast, E1a(r) virus displayed an eightfold increase in the number of foci when compared with *In*340 virus (Table 1). Unlike adenovirus mutants that transform CREF cells at frequencies greater than wild-type virus (1, 21, 33), the foci resulting from E1a(r) virus infection appeared to have the same morphological attributes of *In*340 virus-transformed CREF cells, on the basis of loss of fibroblastic CREF cell morphology. In further contrast to viruses which incompletely transform CREF cells, the E1a(r) virus displayed no cold-sensitive transformation phenotype (Table 2) (5; data not shown). Several independently cloned cell lines isolated from foci established by the E1a(r) virus were analyzed for their ability to grow in soft agar and, like wild-type-virus-transformed cells, did so efficiently at both 32 and 37°C (Table 2). This contrasts with results obtained by using the A2 cell line, which is transformed by the E1A mutant cold-sensitive Ad5hr1 virus (1, 21) and does not proliferate in soft agar at 32°C. Therefore, reducing the rate of E1A and E1B gene expression during CREF cell infection leads to an increase in focus formation, and the foci obtained are indistinguishable from wild-type virus.

TABLE 3. A high rate of viral E1A gene expression can suppress the E1a(r) virus transformation phenotype[a]

Virus	MOI	Genotype[b]	E1A expression (fold)[c]	Mean foci/dish[d]
E1a(r)	10	Reversed E1A	0.01	139.3
E1a(r) + *dl*110	10 + 10	−E1B 495R	1	16.4
E1a(r) + *dl*112	10 + 10	−E1B 175R	1	27
E1a(r) + α3-3	10 + 10	−E1A	0.01	126
*In*340	10	WT	1	16.8
*In*340 + *dl*110	10 + 10	−E1B 495R	2	29.8
*In*340 + *dl*112	10 + 10	−E1B 175R	2	26.2
*In*340 + α3-3	10 + 10	−E1A	1	38.2
Mock				0

[a] CREF cells were infected at the multiplicity of infection (MOI) shown, and following adsorption, cells were suspended and replated at 10^5 cells per 60-mm (diameter) plate for 6 weeks at 37°C.

[b] The orientation of the E1A gene on the viral genome and the E1A mRNAs which are expressed is shown. WT, Wild type.

[c] The rate of E1A gene expression following an 8-h infection of CREF cells at the multiplicity of infection shown for each virus is shown relative to the level observed for *In*340.

[d] Mean number of transformed cell colonies; each value represents the quantitation of 6 to 10 plates.

A high rate of E1A but not E1B gene expression can suppress the transformation phenotype of the E1a(r) virus. To determine which viral gene product(s) had to be expressed at a reduced rate on the E1a(r) virus genome to maintain its transformation phenotype, complementation CREF transformation assays were done. CREF cells were infected with the E1a(r) virus or *In*340 virus at 10 PFU per cell alone or in combination with 10 PFU per cell of the viruses shown in Table 3. The number of foci obtained was determined following 5 weeks of incubation at 37°C. Both E1B deletion viruses H5dl110 (no E1B 55-kilodalton protein) and H5dl112 (no E1B 21-kilodalton protein) express wild-type levels of the E1A mRNAs following CREF cell infection (2, 24; data not shown). These viruses could suppress the E1a(r) virus transformation phenotype (Table 3). Alone these E1B mutant viruses have been shown to be transformation defective (2, 24). Therefore, it is likely that the high rate of E1A gene expression in the coinfected cells was responsible for the loss of the E1a(r) virus supertransforming phenotype. Coinfection of CREF cells with *In*340 virus and these same E1B mutant viruses led to a twofold increase in the number of foci obtained, when compared with *In*340 virus alone (Table 3).

It should be noted that the E1A gene products expressed by the coinfecting E1B deletion mutant viruses do not transactivate the E1B gene on the E1a(r) virus genome (data not shown). As a consequence, during these coinfections, E1B gene expression from the E1a(r) genome remains uniformly low. Therefore, it can be concluded that altered E1B gene expression does not contribute to the transforming phenotype of the E1a(r) virus. This was tested by coinfecting CREF cells with the E1a(r) virus and a virus which expresses wild-type levels of the E1B mRNAs (α3-3) upon CREF cell infection (data not shown). The α3-3 virus had no effect on the E1a(r) virus transformation phenotype (Table 3). Therefore, increasing the rate of E1A gene expression to wild-type levels during a virus coinfection leads to a decrease in the transforming efficiency of the E1a(r) virus.

Both E1A gene products are necessary when the E1A gene is expressed at a low rate. While both E1A proteins can independently initiate unique pathways leading to cellular transformation, both proteins are needed to establish a morphologically complete transformed cell phenotype (33). To determine if reducing the rate of expression of either E1A mRNA would change both the frequency of focus formation and the phenotypes of the transformed cells, mutations were engineered into the E1a(r) virus genotypic background to allow low rates of either E1A 13S or 12S mRNA expression. H2dl1500 virus contains a deletion that prevents cytoplasmic accumulation of the E1A 13S mRNA (Fig. 1). This virus transformed CREF cells at frequencies 5- to 10-fold higher than that of wild-type *In*340 virus (Table 4), and the lack of 289R E1A protein expression resulted in foci that are fibroblastic in appearance (incompletely transformed) and cold sensitive for maintenance of the transformed cell phenotype (33). *pm*975 virus contains a point mutation that prevents accumulation of the E1A 12S mRNA, and this virus transformed CREF cells at a frequency similar to wild-type virus (Table 4), although the foci obtained cannot proliferate in soft agar (33). This virus also has the unique property of being cytotoxic, most probably resulting from a disruption in

TABLE 4. When E1A gene expression is reduced, both E1A gene products are necessary for CREF cell transformation[a]

Virus	MOI	Genotype	E1A expression (fold)	Mean foci/dish (expt 1)	% Clone survival
*In*340	10	WT	1	19.75	90
E1a(r)	1	Reversed E1A		28.25	93
E1a(r)	10	Reversed E1A	0.01	180.3	93
H2dl1500	1	−E1A 13S		23.2	91
H2dl1500	10	−E1A 13S	0.2	142	89
E1a(r)1500	10	Reversed −E1A 13S	0.01	0.6	93
H5*pm*975	10	−E1A 12S	1	12.3	72
E1a(r)975	10	Reversed −E1A 12S	0.01	0.4	93
E1a(r)975 + E1a(r)1500	10 + 10			165	89
Mock				0	94

[a] Procedures were as described in Table 1, footnote *a*. Values were determined as described in Table 1, footnotes *b* to *e*. MOI, Multiplicity of infection; WT, wild type.

cellular gene expression, owing to the *trans*-activating function of the 289R protein (33).

CREF cell infection (Table 4) with viruses that contained the *pm*975 [E1a(r)975] or H2dl1500 [E1a(r)1500] mutations in the E1a(r) virus genome led to the production of no transformed cell foci. This was in contrast to the transforming potentials of these same viral E1A gene mutations when they were expressed at wild-type levels. Therefore, when the E1A gene is expressed at a low rate, both E1A gene products must cooperate to produce the high frequency of cellular transformation observed. This finding was tested by coinfecting CREF cells with the transformation-defective E1a(r)975 and E1a(r)1500 viruses and observing a high frequency in cellular transformation (Table 4). Thus, the two mutations can complement each other in *trans*, confirming the requirement for both E1A gene products. This additionally demonstrates the validity of the coinfection transformation assay.

Transformed cell lines established from E1a(r) virus- and *In*340 virus-infected CREF cells express their E1A and E1B genes at similar levels. Eight independently cloned transformed cell lines were established from foci resulting from E1a(r) virus and *In*340 virus infection of CREF cells. Total cytoplasmic RNA was isolated from all the cell lines, and the endogenous levels of the viral E1A and E1B mRNAs were determined by using SP6-generated ^{32}P-labeled antisense RNA probes. All the cell lines expressed both the E1A and E1B mRNAs, which was predicted since these gene products are necessary for the morphological phenotypes of the transformed cells (Fig. 3). Surprisingly, the accumulation of the viral early gene mRNAs, although variable, was on average similar among the cell lines established by either virus (Fig. 3; a longer exposure of this gel showed low levels of E1A and E1B mRNAs for the E1a(r) CREF clone 5 cell line [data not shown]). This was in sharp contrast to the results observed following E1a(r) and *In*340 virus infection of CREF cells, in which E1A and E1B expression was significantly reduced from that of the E1a(r) viral genome (Fig. 2). Furthermore, there did not appear to be more copies of E1a(r) virus DNA integrated into the host cell DNA, when compared with wild-type virus DNA (data not shown).

A conditionally transformed cell line is equally highly susceptible to transformation by both *In*340 and E1a(r) viruses.

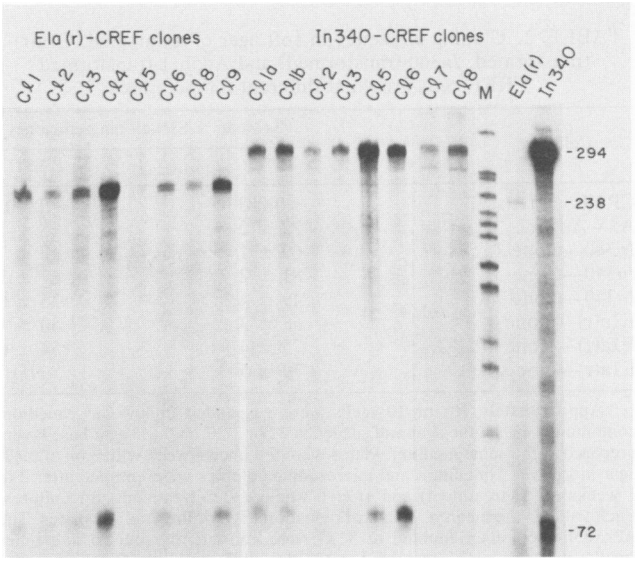

FIG. 3. E1A and E1B gene expression is on the average similar in E1a(r) and *In*340 virus-transformed CREF cell lines. Cytoplasmic RNAs were isolated from the cloned transformed cell lines shown above each lane, and 20 μg was hybridized to the E1A- and E1B-specific antisense RNA probe shown in Fig. 2. To the right of the labeled DNA markers (lane M) are the protected RNAs resulting from assaying 10 μg of total cytoplasmic RNA following infection with E1a(r) virus (20 PFU per cell; 40 h) [lane E1a(r)] and *In*340 virus (5 PFU per cell; 8 h) (lane *In*340).

Since the E1a(r) virus produces low levels of the E1A and E1B mRNAs in infected CREF cells, this allows more cells in the population to become transformed. To test the possibility that transformation requires the selection of a specific cell type in the infected cell population, we used an adenovirus-transformed cell line that has a conditional transformed cell phenotype. At the permissive temperature of 37°C, the A2 cell line (transformed by the Ad5hr1 virus) (5) displays an incomplete transformed cell phenotype. At the nonpermissive temperature of 32°C, the cells revert to a morphology similar to untransformed CREF cells. Since the A2 cell line is an established, partially transformed cell line, we reasoned that it might represent a cell type that was initially selected for some step in cellular transformation. Therefore, this cell line may be more susceptible to transformation by wild-type virus than are normal CREF cells.

A2 cells were maintained at 32°C for 4 weeks prior to

TABLE 5. A conditionally transformed CREF cell line is more susceptible to complete transformation by *In*340 virus than CREF cells at 32°C[a]

Virus	MOI	Genotype	E1A expression (fold)	Mean foci/dish[b]	
				A2	CREF
*In*340	10	WT	1	345	18.2
Ad5hr1	10	−E1A 13S	0.2	3.5	4.2
E1a(r)	10	Reversed E1A	0.01	289	159.2
H5dl118	10	−E1B	1	368	0
α3-3	10	−E1A		0.6	0
Mock				0	0

[a] Procedures were as described in Table 3, footnote *a*. Values were determined as described in Table 3, footnotes *b* to *e*. MOI, Multiplicity of infection; WT, wild type.

[b] Staining in the A2 and CREF cell transformation assays was at 4.5 and 7 weeks, respectively.

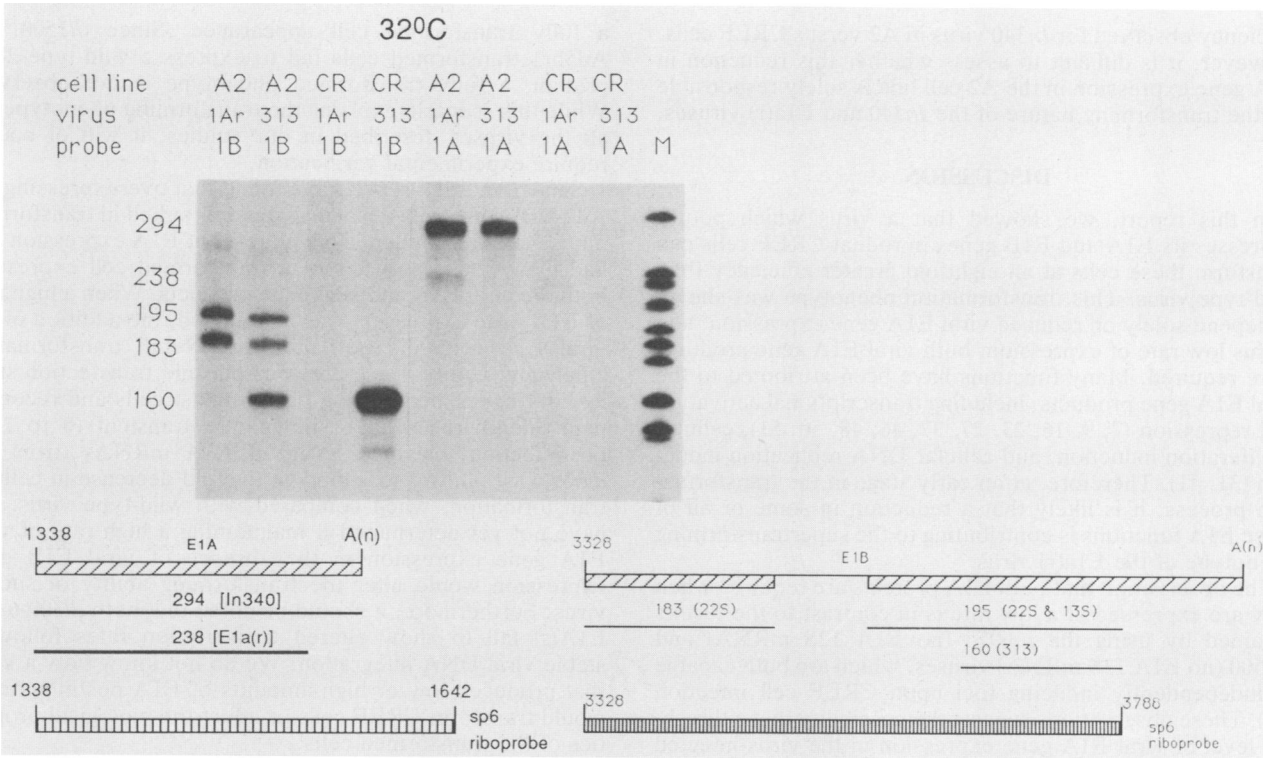

FIG. 4. E1A gene expression is similarly low in E1a(r) virus-infected CREF and A2 cells, while E1A gene expression is reduced in wild-type virus-infected A2 versus CREF cells. A2 and CREF cells were maintained at 32°C for 4 weeks prior to infection by viruses. Cytoplasmic RNAs were isolated from E1a(r) and H5dl313 (27) virus-infected (20 PFU per cell) CREF and A2 cells at 48 h postinfection. Viral E1A and E1B mRNA levels were determined as described in the legend to Fig. 2 and Materials and Methods. Lanes: CR, CREF cells; A2, A2 cells; 1Ar, E1a(r) virus; 313, H5dl313 virus; 1A and 1B, SPG riboprobes (diagramed at the bottom of the figure); M, marker S. Nucleotide numbers are shown to the left of the gel (the 160-nucleotide fragment protected in the SP6-RNase T2 assay corresponds to an E1A-E1B fusion mRNA expressed from the H5dl313 virus genome; this mRNA has been shown to encode functional E1A proteins [27]).

infection by the viruses shown in Table 5. The Ad5hr1 and α3-3 viruses were unable to transform the A2 or CREF cell lines at 32°C, showing the cold-sensitive E1A-defective phenotypes of the A2 cell line and these viruses (Table 2). *In*340 virus transformed the A2 cell line very efficiently, when compared with CREF cells (Table 5). A virus which does not express wild-type E1B gene products (H5dl118) (3) displayed the same transforming properties as *In*340 virus in the A2 cell line, since the endogenous E1B gene products could complement this defect. The E1a(r) virus transformed the A2 cells at the same high efficiency as *In*340 virus. This contrasts with earlier results obtained using the E1a(r) virus to transform CREF cells, whereby reducing the rate of E1A gene expression led to an increase in focus formation. Therefore, following E1a(r) virus infection, either reduced E1A gene expression does not occur in the preselected A2 cell line or, if it does occur, it does not seem to contribute to the transformation frequency.

This was tested by infecting both the A2 and CREF cell lines with the E1a(r) virus (at 20 PFU per cell) at 32°C. Total cytoplasmic RNA was harvested at 48 h postinfection, and prior to viral mRNA analysis, all of the RNA samples were normalized by measuring actin mRNA levels (data not shown). As shown in Fig. 4, E1a(r) viral E1A mRNA accumulation was similar (within twofold) in both the CREF and A2 cell lines (compare the fifth and seventh lanes). Furthermore, E1a(r) virus E1A mRNA accumulation in the A2 cell line was approximately 10-fold less than endogenous E1A mRNA levels from the Ad5hr1 integrated viral genomes. We have previously shown that E1A expression in the A2 cell line is approximately fivefold less than the E1A mRNA levels observed following an 8-h wild-type virus infection (20 PFU per cell) of CREF cells (5; data not shown). Taken together, the overall reduction in E1A gene expression from the E1a(r) virus in both cell lines is roughly 50-fold relative to that from the *In*340 virus. Therefore, the loss of the E1a(r) virus transforming phenotype in the A2 cell line was not the result of altered (increased) expression from the E1A gene promoter.

We next determined whether the A2 cell line supported less exogenous viral E1A gene expression than CREF cells. For this analysis, we used the E1A and E1B gene deletion mutant virus H5dl313 (27, 28). This virus expresses an E1A promoter-driven E1A-E1B fusion transcript that encodes fully functional E1A proteins (27). The H5dl313 E1A gene is fused to the end of the E1B gene so that the probe used to measure E1A mRNA levels is actually homologous to the E1B sequence, and the endogenous (from Ad5hr1 virus) and exogenous (from H5dl313 virus) viral mRNAs can be distinguished. Following a 48-h infection of CREF cells by the H5dl313 virus, large amounts of exogenously expressed E1A mRNAs were observed (Fig. 4, fourth lane; the 160-nucleotide band is specific to H5dl313 E1A mRNA). In A2 cells, the relative amount of E1A mRNA expression from the H5dl313 virus is fivefold depressed (compare the intensity of the 160-nucleotide band in the second and fourth lanes). One might speculate that the reduced levels of E1A mRNAs from the H5dl313 virus contribute to the increased transformation

efficiency observed for *In*340 virus in A2 versus CREF cells. However, it is difficult to assess whether this reduction in E1A gene expression in the A2 cell line is solely responsible for the transforming nature of the *In*340 and E1a(r) viruses.

DISCUSSION

In this report, we showed that a virus which poorly expresses its E1A and E1B genes in rodent CREF cells can transform these cells at an eightfold greater efficiency than wild-type virus. This transformation phenotype was shown to depend solely on reduced viral E1A gene expression, and at this low rate of expression, both viral E1A gene products were required. Many functions have been attributed to the viral E1A gene products, including transcriptional activation and repression (7, 9, 16, 23, 27, 37, 46, 48, 50, 51), cellular proliferation induction, and cellular DNA replication induction (31, 41). Therefore, at an early stage in the transformation process, it is likely that a reduction in some or all of these E1A functions is contributing to the supertransforming phenotype of the E1a(r) virus.

The observation that both E1A proteins are required when they are expressed at a low rate is in contrast to the results obtained by using the *pm*975 (no E1A 12S mRNA) and *dl*1500 (no E1A 13S mRNA) viruses, which are both capable of independently inducing foci upon CREF cell infection (33). These observations suggest the possibility that a threshold level of viral E1A gene expression in the virus-infected cell is required to establish the transformed cell phenotype but that the threshold can vary when both viral E1A gene products are expressed. Others have observed variable requirements for different oncogenes as they shift the levels of expression of the oncogenes (47, 49). These results suggest that complex and possibly multiple pathways must exist which can lead to the generation of the transformed cell.

Like the E1a(r) virus, *dl*1500 virus can transform CREF cells at a greater frequency than *In*340 and *pm*975 viruses. However, unlike the E1a(r) virus-induced foci, the *dl*1500 virus foci remain fibroblastic in appearance (incomplete transformed morphology) and are conditionally transformed. Analysis of viral E1A gene expression in *dl*1500 virus-infected CREF cells has revealed a 5- to 10-fold decrease relative to *In*340 virus, due to the absence of the *trans*-activating protein domain unique to the 13S mRNA gene product (24, 33). Therefore, it is possible that the transformation phenotype of the *dl*1500 virus could be due to the combined absence of viral E1A 13S mRNA expression and a general reduction in the rate of viral E1A transcription.

Taken together, these findings could explain why the E1a(r) virus has certain transforming properties resembling both the *dl*1500 and wild-type viruses. We found that following an 88-h infection of CREF cells by the E1a(r) virus, E1A mRNA levels are equivalent to those observed following a 16-h CREF cell infection by *In*340 virus (Fig. 2C). If we assume that commitment to the transformation process occurs prior to the time when virus-infected cells produce equal levels of E1A mRNAs, we can propose a model. Reduced viral E1A 289R and 243R protein levels in the infected cells somehow could result in more cells participating in this process (as has been postulated for the *dl*1500 virus) (33). Following this cell commitment stage of the process, E1A gene expression gradually increases (possibly from integrated viral genomes) to the point where sufficient E1A 289R protein is expressed and leads to the transactivation of certain viral (E1B) and cellular genes, which results in a fully transformed cell appearance. Since *dl*1500 and Ad5hr1 transformed cells fail to express a wild-type 289R protein, a fully transformed phenotype is not observed. While this scenario explains the transforming phenotypes of all the viruses described in our studies, it will of course require experimental verification.

Senear and Lewis (47) have found that overexpressing the Ad2 E1A gene from a plasmid vector resulted in transformed mouse cells that retained a high level of E1A expression and had many characteristics of a transformed cell expressing both the viral E1A and E1B gene products. When a high rate of E1A gene expression was maintained, the addition of the viral E1B gene did not alter the overall transformation efficiency. Unlike these previous plasmid transfection studies, our experiments using rat fibroblast cells and recombinant adenoviruses have shown that transient (0 to 24 h postinfection) elevated levels of E1A mRNAs (from the *dl*309-6 virus) lead to a modest twofold decrease in cellular transformation, when compared with wild-type virus. We have not yet determined if maintaining a high rate of viral E1A gene expression in the absence of viral E1B gene expression would alter the transforming ability of such a virus. Furthermore, it should be noted that both *dl*309-6 and E1A(r) fail to show altered transcription rates following stable viral DNA integration. We do not know how a virus that produced low or high amounts of E1A postintegration would transform CREF cells or affect the biological properties of the transformed cells.

Lastly, we must consider how the transformed cell regulates viral gene expression following viral DNA integration into the host cell chromosome. Our studies clearly show that viruses which express their E1A and E1B genes at dramatically different rates 8 h after CREF cell infection express comparable levels of E1A and E1B mRNAs in established transformed cell lines (variations in E1A and E1B expression are observed, but the averages of 17 transformed cell lines are the same). There could be several explanations for the regulated viral gene expression observed. First, one might speculate that cells which are ultimately transformed by adenovirus may require localization of the viral DNA in a region of the host DNA sequences that allows cellular dependent *cis* regulation of viral E1A and E1B gene transcription. In general, however there is little evidence that site selection of viral DNA integration contributes to the transforming phenotype for adenovirus (for a review, see reference 12). Second, the cell that is ultimately transformed by adenovirus does not either express or posttranslationally modify certain cellular transcription factors that function to modulate the rate of viral early gene expression. These could include any of the cellular transcriptional factors known to regulate viral E1A and E1B gene expression (for a review, see reference 30) or some novel factor that is uniquely present in the transformed cell. Third, the integrated viral DNA sequences may assume a DNA conformation that allows uniformly low rates of viral E1A and E1B gene expression, despite the level observed from episomal viral DNA templates. This concept is supported by the studies of Gaynor and Berk (15) and our observation that a prolonged E1a(r) virus infection of CREF cells leads to increased levels of the viral E1A and E1B cytoplasmic mRNAs. Therefore, a conformational change in the viral DNA template may be contributing to the rate of viral E1A gene expression.

ACKNOWLEDGMENTS

We thank A. Berk and P. Hearing for viruses used in these studies.

This work was aided by a grant from the American Cancer Society (JFRA-235) and by Public Health Service grant CA48707-10 from the National Institutes of Health to L.E.B. G.R.A. was supported by an American Cancer Society postdoctoral fellowship (PF-02941-03). L.E.B. is a Sinsheimer Scholar.

LITERATURE CITED

1. **Babiss, L. E.** 1989. The cellular transcription factor E2f requires viral E1A and E4 gene products for increased DNA-binding activity and functions to stimulate adenovirus E2A gene expression. J. Virol. **63**:2709–2717.
2. **Babiss, L. E., P. B. Fisher, and H. S. Ginsberg.** 1984. Effect on transformation of mutations in the early region 1b-encoded 21- and 55-kilodalton proteins of adenovirus type 55. J. Virol. **52**:389–395.
3. **Babiss, L. E., and H. S. Ginsberg.** 1984. Adenovirus type 5 early region 1b gene product is required for efficient shutoff of host protein synthesis. J. Virol. **50**:202–212.
4. **Babiss, L. E., H. S. Ginsberg, and J. E. Darnell, Jr.** 1985. Adenovirus E1B proteins are required for accumulation of late viral mRNA and for effects on cellular mRNA translation and transport. Mol. Cell. Biol. **5**:2552–2558.
5. **Babiss, L. E., H. S. Ginsberg, and P. B. Fisher.** 1983. Cold-sensitive expression of transformation by a host range mutant of type 5 adenovirus. Proc. Natl. Acad. Sci. USA **80**:1352–1356.
6. **Barker, D. D., and A. J. Berk.** 1987. Adenovirus proteins from both E1B reading frames are required for transformation of rodent cells by viral infection and DNA transfection. Virology **156**:107–121.
7. **Berk, A. J., F. Lee, T. Harrison, J. Williams, and P. A. Sharp.** 1979. Pre-early adenovirus 5 genome product regulates synthesis of early viral messenger RNAs. Cell **17**:935–944.
8. **Berk, A. J., and P. A. Sharp.** 1978. Structure of adenovirus 2 early mRNAs. Cell **14**:695–711.
9. **Borelli, E., R. Hen, and P. Chambon.** 1984. Adenovirus-2 E1A products repress enhancer-induced stimulation of transcription. Nature (London) **312**:608–612.
10. **Chang, E. H., M. E. Furth, E. M. Scolnick, and D. R. Lowry.** 1982. Tumorigenic transformation of mammalian cells induced by a normal human gene homologous to the oncogene of Harvey murine sarcoma virus. Nature (London) **297**:479–483.
11. **Chow, L. T., T. R. Broker, and J. B. Lewis.** 1979. Complex splicing patterns of RNAs from the early regions of adenovirus-2. J. Mol. Biol. **134**:265–303.
12. **Doerfler, W.** 1982. Uptake, fixation and expression of foreign DNA in mammalian cells: the organization of integrated adenovirus DNA sequences, p. 127–194. *In* T. Graf and R. Jaenisch (ed.), Tumorviruses, neoplastic transformation and differentiation. Springer-Verlag, New York.
13. **Fisher, P. B., L. E. Babiss, I. B. Weinstein, and H. S. Ginsberg.** 1982. Analysis of type 5 adenovirus transformation with a cloned rat embryo cell line (CREF). Proc. Natl. Acad. Sci. USA **79**:3527–3531.
14. **Friedman, J. M., L. E. Babiss, D. F. Clayton, and J. E. Darnell, Jr.** 1986. Cellular promoters incorporated into the adenovirus genome: cell specificity of albumin and immunoglobulin gene expression. Mol. Cell. Biol. **6**:3791–3797.
15. **Gaynor, R. B., and A. J. Berk.** 1983. cis-induction of adenovirus transcription. Cell **33**:683–693.
16. **Gaynor, R. B., D. Hillman, and A. J. Berk.** 1984. Adenovirus early region 1A protein activates transcription of a non viral gene introduced into mammalian cells by infection or transfection. Proc. Natl. Acad. Sci. USA **81**:1193–1197.
17. **Ginsberg, H. S.** 1984. Adenoviruses. Plenum Publishing Corp., New York.
18. **Graham, F. L., J. Smiley, W. C. Russell, and R. Nairu.** 1977. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J. Gen. Virol. **36**:59–77.
19. **Haley, K. P., G. Overhouseer, L. E. Babiss, H. S. Ginsberg, and N. C. Jones.** 1984. Transformation properties of type 5 adenovirus mutants that differentially express the E1A gene products. Proc. Natl. Acad. Sci. USA **81**:5734–5738.
20. **Harlow, E., P. Whyte, B. R. Franza, Jr., and C. Schley.** 1986. Association of adenovirus early region 1A proteins with cellular polypeptides. Mol. Cell. Biol. **6**:1579–1589.
21. **Harrison, T., F. L. Graham, and J. Williams.** 1977. Host range mutants of adenovirus type 5 defective for growth in HeLa cells. Virology **77**:319–329.
22. **Hearing, P., and T. Shenk.** 1983. The adenovirus type 5 E1A transcriptional control region contains a duplicated enhancer element. Cell **33**:695–703.
23. **Hen, R., E. Borrelli, and P. Chambon.** 1985. Repression of the immunoglobulin heavy chain enhancer by the adenovirus-2 E1a products. Science **230**:1391–1394.
24. **Herbst, R. S., H. Hermo, P. B. Fisher, and L. E. Babiss.** 1988. Regulation of adenovirus and cellular gene expression and of cellular transformation by the E1b-encoded 175-amino acid protein. J. Virol. **62**:4634–4643.
25. **Herbst, R. S., M. Pelletier, E. M. Boczko, and L. E. Babiss.** 1990. The state of cellular differentiation determines the activity of the adenovirus E1A enhancer element: evidence for negative regulation of enhancer function. J. Virol. **64**:161–172.
26. **Houweling, A., P. J. van den Elsen, and A. J. Van der Eb.** 1980. Partial transformation of primary rat cells by the left-most 4.5% fragment of adenovirus 5 DNA. Virology **105**:537–550.
27. **Jones, N., and T. Shenk.** 1979. An adenovirus type 5 early gene function regulates expression of other early viral genes. Proc. Natl. Acad. Sci. USA **76**:3665–3669.
28. **Jones, N., and T. Shenk.** 1979. Isolation of adenovirus type 5 host range deletion mutants defective for transformation in rat embryo cells. Cell **17**:683–689.
29. **Kitchingman, G. R., and H. Westphal.** 1980. The structure of adenovirus 2 early nuclear and cytoplasmic RNAs. J. Mol. Biol. **137**:23–48.
30. **Lillie, J. W., M. Green, and M. R. Green.** 1986. An adenovirus E1a protein region required for transformation and transcriptional repression. Cell **46**:1043–1051.
31. **Lillie, J. W., P. M. Loewenstein, M. R. Green, and M. Green.** 1987. Functional domains of adenovirus type 5 E1a proteins. Cell **50**:1091–1100.
32. **Miller, A. D., T. Curran, and I. M. Verma.** 1984. c-fos protein can induce cellular transformation: a novel mechanism of activation of a cellular oncogene. Cell **36**:51–60.
33. **Montell, C., G. Courtois, C. Eng, and A. Berk.** 1984. Complete transformation by adenovirus 2 requires both E1A proteins. Cell **36**:951–961.
34. **Montell, C., E. F. Fisher, M. H. Caruthers, and A. J. Berk.** 1982. Resolving the functions of overlapping viral genes by site specific mutagenesis at a mRNA splice site. Nature (London) **295**:380–384.
35. **Moran, E., T. Grodzicker, R. J. Roberts, M. B. Mathews, and B. Zerler.** 1986. Lytic and transforming functions of individual products of the adenovirus E1A gene. J. Virol. **57**:765–775.
36. **Moran, B., and B. Zerler.** 1988. Interactions between cell growth-regulating domains in the products of the adenovirus E1A oncogene. Mol. Cell. Biol. **8**:1756–1764.
37. **Nevins, J. R.** 1981. Mechanism of activation of early viral transcription by the adenovirus E1A gene product. Cell **26**: 213–220.
38. **Oskarsson, M., M. McClements, W. Blair, D. G. Maizel, and G. Vande Woude.** 1980. Properties of normal mouse cell DNA sequence (*sarc*) homologous to the *src* sequence of Maloney sarcoma virus. Science **207**:1222–1224.
39. **Pericaudet, M., G. Akusjarvi, A. Virtanen, and U. Pettersson.** 1979. Structure of two spliced mRNAs from the transforming region of human subgroup C adenoviruses. Nature (London) **281**:694–696.
40. **Pilder, S., J. Logan, and T. Shenk.** 1984. Deletion of the gene encoding the adenovirus 5 early region 1B 21,000-molecular-weight polypeptide leads to degradation of viral and cellular DNA. J. Virol. **52**:664–671.
41. **Quinlan, M. P., and T. Grodzicker.** 1987. Adenovirus E1A 12S protein induces DNA synthesis and proliferation in primary epithelial cells in both the presence and absence of serum. J. Virol. **61**:673–682.
42. **Raptis, L., H. Lamfrom, and T. L. Benjamin.** 1985. Regulation

of cellular phenotype and expression of polyomavirus middle T antigen expression in rat fibroblasts. Mol. Cell. Biol. **5**:2476–2485.

43. **Ricciardi, R. P., R. L. Jones, C. L. Cepko, P. A. Sharp, and B. E. Roberts.** 1981. Expression of early adenovirus genes requires a viral encoded acidic polypeptide. Proc. Natl. Acad. Sci. USA **78**:6121–6125.
44. **Ruley, H. H.** 1983. Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture. Nature (London) **304**:602–606.
45. **Sarnow, P., Y. Shih Ho, J. Williams, and A. J. Levine.** 1982. Adenovirus E1b-58kd tumor antigen and SV40 tumor antigen are physically associated with the same 54 kd cellular protein in transformed cells. Cell **28**:387–394.
46. **Schrier, P. I., R. Bernards, R. T. M. J. Vaessen, A. Houweling, and A. J. Van der Eb.** 1983. Expression of class I major histocompatability antigens switched off by highly oncogenic adenovirus 12 in transformed rat cells. Nature (London) **305**: 771–775.
47. **Senear, A. W., and J. B. Lewis.** 1986. Morphological transformation of established rodent cell lines by high-level expression of the adenovirus type 2 E1a gene. Mol. Cell. Biol. **6**:1253–1260.
48. **Simon, M. C., T. M. Fisch, B. J. Benecke, J. R. Nevins, and N. Heintz.** 1988. Definition of multiple, functionally distinct TATA elements, one of which is a target in the hsp70 promoter for E1A regulation. Cell **52**:723–729.
49. **Spandidos, D. A., and N. M. Wilkie.** 1984. Malignant transformation of early passage rodent cells by a single mutated human oncogene. Nature (London) **310**:469–475.
50. **Stein, R., and E. B. Ziff.** 1984. HeLa cells β-tubulin gene transcription is stimulated by adenovirus 5 in parallel with viral early genes by an E1a-dependent mechanism. Mol. Cell. Biol. **4**:2792–2801.
51. **Velcich, A., and E. Ziff.** 1985. Adenovirus E1A proteins repress transcription from the SV40 early promoter. Nature (London) **292**:595–600.
52. **White, E., and R. Cipriani.** 1989. Specific interrpution of intermediate filaments and the nuclear lamina by the 19-kilodalton product of the adenovirus E1B oncogene. Proc. Natl. Acad. Sci. USA **86**:9886–9890.
53. **White, E., and R. Cipriani.** 1990. Role of adenovirus E1B proteins in transformation: altered organization of intermediate filaments in transformed cells that express the 19-kilodalton protein. Mol. Cell. Biol. **10**:120–130.
54. **Whyte, P., J. J. Buchkovich, J. M. Horowitz, S. H. Friend, M. Raybuck, R. A. Weinberg, and E. Harlow.** 1988. Association between an oncogene and anti-oncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product. Nature (London) **334**:124–129.
55. **Whyte, P., N. M. Williamson, and E. Harlow.** 1989. Cellular targets for transformation by the adenovirus E1A proteins. Cell **56**:67–75.
56. **Yee, S., and P. E. Branton.** 1985. Detection of cellular proteins associated with human adenovirus type 5 early region 1A polypeptides. Virology **147**:142–153.
57. **Zerler, B., E. Moran, K. Maruyama, J. Moomaw, T. Grodzicker, and H. E. Ruley.** 1986. Adenovirus E1A coding sequences that enable *ras* and *pmt* oncogenes to transform cultured primary cells. Mol. Cell. Biol. **6**:887–899.