Role of the Adenovirus E1B 19,000-Dalton Tumor Antigen in Regulating Early Gene Expression

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Received 18 December 1987/Accepted 16 May 1988

Mutations in the adenovirus gene encoding the E1B 19-kilodalton protein (the 19K protein) result in pleiotropic phenotypes that affect the host cell and virus growth. Examination of viral gene expression in HeLa cells infected with E1B 19K mutant viruses revealed synthesis and accumulation of E1A proteins to higher steady-state levels than those proteins synthesized during infection with the wild-type virus. As a consequence of elevated E1A levels, another early gene product, the 72K DNA-binding protein, accumulated earlier in mutant-infected cells. In a 12S E1A cDNA virus background, E1B 19K gene mutations had a more profound effect. Larger amounts of the 12S E1A product were present in E1B mutant-infected cells. A deletion mutation that eliminated expression of the 19K protein was also responsible for a 200-fold increased plaque-forming efficiency of the 12S cDNA virus in HeLa cells and an increased rate of virus production. Therefore, the E1B 19K tumor antigen may function to down-regulate virus replication by repressing E1A-dependent gene transcription. Eliminating expression of the E1A 13S and 12S gene products by substitution of an E1A 9S cDNA gene, however, uncovered a stimulatory effect of the E1B 19K protein on early gene expression and virus replication. An E1A 9S virus with a wild-type gene encoding the E1B 19K protein displayed increased early gene transcription, synthesized more 72K DNA-binding protein, and replicated more efficiently than an E1A 9S virus containing a mutation that eliminated expression of the 19K protein. Therefore, the E1B 19K protein has both positive and negative effects on early gene expression and virus replication. In the presence of functional E1A gene products, the 19K protein repressed E1A-dependent gene expression, but in the absence of E1A, the 19K protein stimulated viral gene expression and DNA synthesis. This raises the possibility that the E1B 19K protein functions to repress transcription by modifying the activity of the E1A proteins. Independent of E1A, however, the E1B 19K protein can increase viral gene expression and DNA synthesis, which then leads to increased virus replication.

Early region 1 (E1) of the adenovirus genome is primarily responsible for regulating the expression of adenovirus genes during infection (reviewed in reference 4). Induction of cellular transformation also requires E1 expression (reviewed in references 7 and 12). E1 consists of two distinct transcription units, designated E1A and E1B. Although the E1A gene products have been shown to play a predominant role in both transactivation of adenovirus gene expression and transformation, proper regulation of gene expression and induction of the full transformed phenotype requires expression of the E1B gene (16, 17, 29, 33, 58, 73).

The E1A gene encodes five mRNAs, the 13S, 12S, 11S, 10S, and 9S products, which are derived by alternative splicing (Fig. 1). Many functions of E1A have been attributed to the individual E1A products through the characterization of adenovirus mutants capable of expressing specific E1A mRNAs in place of genomic E1A sequences (22, 47, 48, 65, 71, 81). Furthermore, the E1A coding region has been subdivided, based on amino acid homology between adenovirus serotypes, into three conserved domains (Fig. 1) (44, 49, 50). Various combinations of domains are expressed in the different E1A gene products depending on the location of the splice sites.

The 13S mRNA encodes a 289-amino-acid protein which includes all three functional domains (Fig. 1) and efficiently activates the transcription of adenovirus promoters, including the E1A promoter, as well as some cellular promoters (14, 30, 35, 59, 64). Viruses expressing solely the 13S E1A

gene product are capable of productive infection (47, 48, 81), although the 13S virus is somewhat defective for replication in quiescent cells (47, 62). Expression of the 13S mRNA product is sufficient for cooperation with the *ras* oncogene to transform primary cells (84).

The 12S mRNA encodes a 243-amino-acid protein which is missing 46 internal amino acids of conserved domain 3 (Fig. 1). The 12S gene product is therefore deficient in transcriptional activation, a function ascribed to domain 3, and viruses which express solely the 12S gene product are defective for productive infection in HeLa cells (47, 48). The 12S E1A product, however, still retains the ability to immortalize primary cells in culture (22, 47, 48) and is capable of stimulating cellular DNA synthesis when introduced into quiescent cells (34, 63).

The 9S mRNA has the capacity to encode a 55-amino-acid polypeptide (Fig. 1); however, no gene product has been observed in infected cells. The 9S E1A gene product has no detectable activity in regulating gene expression, transactivation, or transformation. As a consequence, a 9S cDNA virus is defective for productive infection and transformation (22, 84).

The more recently identified E1A 11S and 10S mRNAs encode proteins related to the 13S and 12S products, respectively, except that both are missing internal amino acids within conserved domain 1 (Fig. 1) (65, 71). The activity of the 11S and 10S gene products probably represents a subset of the activity of the 13S and 12S gene products, since eliminating the expression of 11S and 10S mRNAs in genomic 13S or 12S cDNA viruses does not have a major effect

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FIG. 1. Schematic representation of E1 transcripts and polypeptides. Thin arrows indicate differentially spliced mRNAs, with carets marking introns removed by alternative splicing. Boxes delineate protein-coding regions: ■, protein products related to the E1A 13S gene product; ▨, protein products related to the E1B 55K protein. A more comprehensive presentation of E1B 55K-related protein products can be found in reference 42. Conserved domains 1, 2, and 3 within the E1A coding region are marked. Numbers indicate base pairs, beginning from the left end of the adenovirus genome.

on virus replication or transformation (50, 71; C. Stevens, personal communication).

The E1A proteins have also been shown to repress transcription that is dependent on cellular and viral enhancers, including the E1A enhancer (6, 21, 26, 27, 57, 74, 75). Therefore, the E1A gene products are potentially capable of both activating and repressing their own transcription. From mutational analysis of the E1A gene, the enhancer repression activity appears to reside within domains 1 and 2, shared by both 13S and 12S gene products (43, 60).

In contrast to E1A, the function of the E1B gene products during productive infection and transformation is less clear. The E1B region encodes two major tumor antigens, the E1B 19,000-dalton protein (the 19K protein) and the 55K protein (Fig. 1) (reviewed in reference 66). As with the E1A gene, much of the functional information regarding E1B has been derived from the characterization of adenovirus mutants. These studies have implicated the E1B 55K protein in the transport and/or processing of adenovirus late mRNA (2, 53, 55, 80), as well as the shutoff of host cell protein synthesis (2). Adenovirus mutants with mutations in the genes encoding both the E1B 55K and the 19K proteins (the 55K and 19K genes) are also defective for transformation (3, 5, 8, 13, 33, 53, 54, 68–70, 78).

Adenovirus mutants containing lesions in the E1B 19K gene have very pleiotropic phenotypes. The first, and most dramatic, of the phenotypes that these mutants induced on infection is the degradation of host cell chromosomal and viral DNA (*deg* phenotype) (9, 10, 38, 54, 78). Therefore, a main function of the E1B 19K protein is to protect or stabilize both viral and cellular DNA during infection. Second, infection with an E1B 19K gene mutant virus induces an enhanced and abnormal cytopathic effect (*cyt*, or cytocidal phenotype) (54, 67–70, 78). Third, E1B 19K mutants form large plaques (*lp* phenotype) (8, 67, 68, 78). Fourth, E1B 19K mutant viruses possess an unusual host range (*hr* phenotype, in that mutant viruses have a substantial growth advantage over the wild-type virus in human W138 cells (77, 79). Increased viral gene transcription appears to be responsible for the elevated replication in these cells of E1B viruses with mutations in the 19K gene (77). These data raised the possibility that the E1B 19K protein functions to repress early gene expression during infection. Furthermore, all the phenotypes attributed to the E1B 19K gene, including the *hr* phenotype, are E1A dependent, beyond the requirement for E1A to activate transcription, since infection with a 9S virus carrying a 19K gene point mutation did not induce *cyt*, *deg*,

lp, and *hr* phenotypes even at high multiplicities of infection (77, 79). This suggested a functional interaction between E1A and E1B 19K gene products.

The mechanism by which the E1B 19K gene product regulates gene expression, the involvement of the E1A proteins, and the ways in which this might lead to the manifestation of the *cyt*, *deg*, *lp*, and *hr* phenotypes are all of great interest. We have examined the effect of E1B 19K gene mutations on early gene transcription in HeLa cells and found similar results to those in W138 cells. That is, in the presence of functional E1A gene products, E1B 19K gene mutations stimulated gene expression, consistent with the previously proposed role of this E1B protein down-regulating adenovirus gene expression during productive infection (77). The consequences of E1B 19K gene mutations are aberrant temporal regulation of viral gene expression and may explain the enhanced *cyt*, *lp*, and *hr* phenotypes observed in mutant-infected cells. In the absence of functional E1A products, however, E1B 19K gene mutations caused a diminution in virus replication. From these results it appears that the E1B 19K protein is also capable of stimulating viral gene expression and DNA synthesis, which is uncovered only with the elimination of E1A protein function.

MATERIALS AND METHODS

Cells and viruses. Human HeLa cells were grown on monolayer culture in Dulbecco modified Eagle medium with 10% fetal bovine serum. 293 cells (18), which express adenovirus E1 gene products, were grown in Dulbecco modified Eagle medium with 10% calf serum. Adenovirus mutants containing E1A 13S, 12S, and 9S cDNA genes in place of genomic E1A sequences (48, 84) were obtained from E. Moran and B. Zerler, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. Point mutations of the E1B 19K gene were introduced into E1A cDNA viruses by overlap recombination between plasmids containing E1A cDNA genes and E1B 19K gene mutations with adenovirus type 5 *dl309* DNA (79). The resulting viruses, 13S.R2, 12S.R2, and 9S.R2, (79) express the appropriate E1A cDNA gene product and in addition carry a point mutation which results in an amino acid substitution (Ser to Asn) in the E1B 19K protein at residue 20 (78). A virus containing an E1A 12S cDNA gene and an E1B 19K gene deletion (12S.337) was constructed by *in vitro* ligation of 12S E1A sequences from 0 to 3.8 map units (*Xba*I site) with 3.8 to 100 map units of DNA from adenovirus type 5 *dl337* followed by transfection of 293

FIG. 2. Time course of expression of early proteins in wild-type *dl309*- and mutant *dl337*-infected cells. HeLa cells were infected with a multiplicity of infection of 20 PFU per cell, and at the given times postinfection (hpi), cells were harvested and protein extracts were analyzed in Western blots for expression of the E1A, E1B 19K, and E2 72K DBP proteins. The amount of the specific antigens was quantitated and is expressed as 10^2 counts per minute.

cells. The mutant with a deletion mutation in the E1B 19K gene, adenovirus type 5 *dl337*, was obtained from T. Shenk, Princeton University, Princeton, N.J., and is missing sequences between nucleotides 1769 and 1912 (54). All viruses were grown in 293 cells, and titers were determined by plaque assays on 293 cells. Virus stocks were stored at -20°C as crude cell lysates. All viruses were derived from adenovirus type 5 *dl309* (32), and this virus was used as the wild-type strain.

Electrophoresis and immunoblot analysis. Monolayers of infected cells were harvested, washed with phosphate-buffered saline, and lysed in sample buffer (37). Samples were subjected to electrophoresis through 10 or 15% polyacrylamide-sodium dodecyl sulfate gels. Immunoblot (Western) analysis was carried out as previously described (78). Monoclonal antibodies directed against the E1A proteins (24) were obtained from E. Harlow, Cold Spring Harbor Laboratory. Rabbit polyclonal antibodies directed against the E1B 19K and the E2A 72K DNA-binding protein (DBP) (77) were used to detect these proteins in extracts from infected cells. Hexon-specific antibodies were obtained from C. Anderson, Brookhaven National Laboratory, Upton, N.Y. Immunological detection of antigens was accomplished with ^{125}I -labeled protein A or ^{125}I -labeled rabbit anti-mouse immunoglobulin G. Immunoreactive products were quantitated by excising radioactive bands followed by counting in a gamma counter.

Southern hybridization of viral DNA. Viral DNA was isolated from infected cells by the method of Hirt (28) with minor modifications (78). DNA was digested with restriction enzymes, subjected to electrophoresis on 1% agarose gels, and transferred to nitrocellulose. Hybridization of filters to labeled adenovirus DNA probe was carried out as previously described (76).

Transcription and RNA analysis. Nuclei from monolayer-infected cells were isolated, and run-on transcription reactions (19, 20) with $[\alpha\text{-}^{32}\text{P}]\text{UTP}$ were carried out as previously described (77). Equal amounts of labeled RNA transcripts were hybridized to denatured adenovirus DNA probes (77). Northern (RNA) analysis of cytoplasmic RNA involved the use of SP6 RNA probes specific for adenovirus early genes (77).

RESULTS

Expression of early gene products in wild-type- and E1B mutant-infected HeLa cells. During infection of human WI38 cells, viruses with mutations in the E1B 19K gene produce more virus, synthesize larger amounts of viral DNA and proteins, and display increased viral gene transcription than wild-type viruses do (77, 79). Surprisingly, the E1B mutant viruses do not cause cell DNA degradation or enhanced cytopathic effect in WI38 cells (77). We investigated the possibility that a similar *hr* phenotype for viral gene expression might also be observed during a HeLa cell infection in which the *deg* and *cyt* phenotypes are apparent. HeLa cells were infected with the wild type virus (*dl309*) or mutant virus with a mutation in the E1B 19K gene (*dl337*), and at the indicated times postinfection cell extracts were analyzed on Western blots for the expression of the E1A and E1B 19K proteins and the 72K DBP (Fig. 2). In wild-type virus-infected HeLa cells, the E1A proteins accumulated early and plateaued (Fig. 2A). In contrast, the E1B mutant-infected cells accumulated higher levels of the E1A proteins, although the onset of the appearance of the E1A products was slightly delayed (Fig. 2B). The overexpression of the E1A proteins occurred primarily after 16 h postinfection, which would correspond to the late phase of infection after the onset of viral DNA synthesis. Similar overexpression of the E1A proteins was observed when infected cells were labeled *in vivo* with ^{35}S methionine and the E1A proteins were immunoprecipitated with E1A-specific monoclonal antibodies (data not shown). A greater difference in the levels of the 72K DBP was observed between *dl309*- and *dl337*-infected cells, with the DBP in mutant-infected cells accumulating to four times the amount observed in cells infected with the wild-type virus (Fig. 2). Late proteins such as hexon also accumulated at earlier times in *dl337*-infected cells (data not shown). Furthermore, the overexpression of the E1A and 72K proteins in mutant-infected cells coincides with the time postinfection that the E1B 19K protein is normally synthesized during the wild-type infection (Fig. 2). By 36 h postinfection, the *cyt* phenotype was apparent in *dl337*-infected cells and cell death was beginning to occur.

Not surprisingly, one consequence of elevated E1A levels in mutant-infected HeLa cells was increased accumulation of early mRNA and early gene transcription, measured by Northern analysis and nuclear run-on assays (data not shown). Higher transcription levels were also the initial cause of the elevated accumulation of early proteins and accelerated infection by *dl337* in WI38 cells (77). This increased transcription in mutant-infected cells is compounded by increased template copy number arising from the accelerated infection (see below). Thus, it appears that the *hr* phenotype observed in mutant virus-infected WI38 cells is also observed in HeLa cells, although to a lesser degree.

Effects of E1B 19K gene mutations on expression of adenovirus early genes in 12S E1A cDNA background. The difference between early gene expression after infection of HeLa cells with *dl309* and *dl337* was obtained only in careful time course experiments and is not nearly as great as that observed in WI38 cells (77). This may be due to the intrinsic permissivity of HeLa cells for adenovirus infection, with the infection progressing to completion rapidly since neither *dl309* or *dl337* is defective for growth. For this reason, we examined the effect of E1B 19K gene mutations in the genetic background of a functionally compromised virus.

The 12S E1A gene product is deficient in transactivating the transcription of early genes owing to the removal of the 13S unique region by alternative splicing. Therefore, the 12S E1A virus is defective in HeLa cells at low multiplicities of infection, but can grow at high multiplicities of infection (48, 79). The effects of E1B 19K gene point and deletion mutations on early gene expression were determined by Western blot analysis in HeLa cells infected at low multiplicities of infection with the 12S E1A cDNA virus. As with a genomic E1A virus, both the E1B 19K gene point (12S.R2) and deletion (12S.337) mutations caused increased accumulation of the 12S E1A protein and 72K DBP during viral infection (Fig. 3). The 19K gene mutations produced a larger effect on E1A and DBP expression in 12S virus-infected cells than in wild-type-infected cells. This may be because transcription of the early genes in wild-type-infected cells is already near maximal, as opposed to the 12S virus, in which early gene transcription is well below maximal.

The elevation in early gene expression observed in the 12S E1A-E1B 19K double-mutant viruses was due in part to template effects. There was more viral DNA replication in the 12S.R2- and 12S.337-infected cells than in comparable infections with a 12S virus containing a wild-type E1B gene (Fig. 4A and B) (79). The 12S.337 virus replicated far more viral DNA than the 12S virus did, despite the pronounced *deg* phenotype, which caused the smear of adenovirus DNA observed in the lanes of mutant-infected cells (Fig. 4A and B).

The stimulation of gene expression resulting from the introduction of an E1B 19K gene mutation into a 12S E1A virus was, however, still observed when viral DNA synthesis was blocked with hydroxyurea, although to a much lesser degree (Fig. 5). The 12S virus expressed very low levels of the DBP by 48 h postinfection, but by 72 h the levels did not increase (Fig. 5). In some cases we observed that the levels of the DBP decreased by 72 h postinfection, which may reflect repression of the E2 early promoter by the 12S E1A protein reported by Rossini (57) and Guilfoyle et al. (21). In contrast, expression of the DBP in 12S.R2-infected cells increased steadily with time in the presence of hydroxyurea to levels far higher than those in 12S-infected cells (Fig. 5). Viral DNA template levels were monitored in infected cells

FIG. 3. Western blot analysis of 12S and 9S virus-infected cells. HeLa cells were infected with 12S or 12S.R2, 12S or 12S.337, and 9S or 9S.R2 at multiplicity of infection of 10 PFU per cell. At the indicated times postinfection (hpi) extracts were made and analyzed in Western blots with antibodies specific for the E1A and E2 72K proteins. Lanes M, mock infected.

in the presence of hydroxyurea by Southern hybridization and found to be only two- or threefold different from each other (data not shown). This indicates that the initial effect of the E1B 19K gene mutations is to increase viral gene expression, independent of viral DNA synthesis, although we cannot rule out the possibility that small differences in template levels can cause very large differences in expres-

FIG. 4. Analysis of viral DNA template levels during viral infection. HeLa cells were infected with 12S and 12S.R2 (A), 12S and 12S.337 (B), or 9S and 9S.R2 (C) as described in the legend to Fig. 3. At the indicated times postinfection (hpi), viral DNA was isolated by Hirt extraction, digested with *Hind*III, and analyzed in Southern blots with labeled adenovirus DNA probe. Lanes M, mock infected.

FIG. 5. Western blot analysis of 72K DBP expression in cells infected in the presence of hydroxyurea. HeLa cells were infected with 12S and 12S.R2 (upper panel) or 9S and 9S.R2 (lower panel) at a multiplicity of infection of 10 PFU per cell and analyzed at the indicated times postinfection (hpi) for expression of the 72K DBP as described in the legend to Fig. 2, except that hydroxyurea (HU; 10 mM) was added at the time of infection. Lanes M, mock infected.

sion of the DBP. Most probably, the elevated levels of gene expression produce increased viral DNA replication, which, in turn, results in more templates for transcription and a still greater increase in gene expression.

Effects of E1B 19K gene mutations on viral gene expression in the absence of functional E1A proteins. To assess the dependence of the E1B 19K gene mutant phenotypes on the E1A proteins, we examined the effect of E1B mutations on gene expression in adenovirus mutants containing a non-functional 9S E1A cDNA gene in place of the genomic sequences. Transcription of adenovirus early genes is very inefficient in the absence of functional E1A gene products during viral infection at low multiplicities of infection (32), but it is possible to detect synthesis of the 72K DBP by Western blot analysis at 48 and 72 h postinfection of HeLa cells with small amounts of a 9S E1A cDNA virus (Fig. 3). In contrast to viruses expressing functional E1A proteins, E1B 19K gene mutations cause a diminution in expression of the 72K DBP in a 9S E1A cDNA virus infection (Fig. 3). The 72K DBP is detectable only at 72 h postinfection in 9S.R2-infected cells (Fig. 3). Thus, it appears that the 19K protein can cause increased gene expression in the absence of functional E1A proteins.

Since E1B 19K gene mutations compromised the expression of the 72K DBP in the absence of functional E1A, we examined whether this reflected differences in the levels of early gene transcription in 9S- and 9S.R2-infected HeLa cells. Transcription of all early genes was reduced in the E1B mutant-infected cells at all times postinfection when compared with transcription in cells infected with the 9S virus (Fig. 6). However, again, the elevation of transcription could be due to template copy number differences. Therefore, the levels of viral DNA replication in the 9S virus-infected cells were examined to determine the contribution of the viral template copy number to the effects on gene transcription.

There was far more viral DNA synthesis in 9S- than 9S.R2-infected cells, which could account for the increased transcription of the DBP (Fig. 4C). To determine whether increased viral DNA replication in 9S virus-infected cells could solely account for the increased expression of the DBP in 9S-infected cells, we examined gene expression under

FIG. 6. In vitro run-on transcription analysis of 9S- and 9S.R2-infected HeLa cells. Nuclei from cells infected with the 9S or 9S.R2 viruses at 50 PFU per cell were isolated at the indicated times postinfection (hpi) and labeled in vitro, and the transcription products were hybridized to immobilized, denatured adenovirus DNA probes specific for each of the early gene regions (shown in lower left panel).

conditions when viral DNA synthesis was blocked. The reduction in gene expression observed in 9S.R2-infected cells was predominantly due to decreased viral DNA synthesis, since the difference in expression of the DBP in 9S- and 9S.R2-infected cells was virtually eliminated by the addition of hydroxyurea at the time of infection (Fig. 5). There was a small difference in expression of the DBP observed at 48 h postinfection, but by 72 h the levels of expression of the DBP in 9S- and 9S.R2-infected cells were comparable (Fig. 5). We have monitored viral DNA template levels in 9S- and 9S.R2-infected cells in the presence of hydroxyurea by Southern hybridization and found them to be virtually identical (data not shown). Therefore, this very small difference between 9S and 9S.R2 at 48 h could represent a small, bona fide effect of the 19K protein stimulating gene expression, but the difference in DBP expression between the two viruses is so small under these conditions that it would be difficult to determine.

Plaque-forming ability of mutant viruses on HeLa and 293 cells. The plaque-forming efficiency of E1B mutant viruses in HeLa cells was examined and compared with that in 293 cells, which complement E1 mutations by supplying the gene products in *trans* (18). No difference in the plaque-forming efficiency was observed between *dl309* and *dl337* in HeLa and 293 cells (Table 1). This was expected, since neither virus is defective for growth in HeLa cells (54). Viruses expressing solely the 12S E1A protein are defective for plaque-forming ability in HeLa cells (47, 48). E1A 12S viruses possessing point or deletion mutations in the E1B 19K gene, however, form plaques in HeLa cells at 7 times (12S.R2) and 275 times (12S.337) the level of a 12S virus with a wild-type E1B gene (Table 1). In contrast, introduction of an E1B 19K gene mutation into a 9S E1A virus resulted in a 60-fold reduction in the plaque-forming efficiency in HeLa cells (Table 1).

E1B mutations have also been shown to cause increased plaque size, which has been designated the large-plaque (*lp*) phenotype (8). Mutants *dl337*, 12S.R2, and 12S.337 all form large plaques in HeLa cells in comparison with their wild-type E1B counterparts, *dl309* and the 12S cDNA virus (Table 1). 9S.R2, however, formed even smaller plaques than the 9S virus did (data not shown), indicating that expression of E1A proteins is also required for expression of the *lp* phenotype. These results also suggest that there may

TABLE 1. Plaque-forming ability of mutant viruses

Virus	Gene composition ^a		Infectivity (PFU/ml)		Infectivity ratio (HeLa/293)
	E1A	E1B	293 cells	HeLa cells ^b	
<i>dl309</i>	WT	WT	9.8×10^8	7.0×10^8	7.1×10^{-1}
<i>dl337</i>	WT	19K ⁻	1.7×10^9	2.5×10^9 *	1.4×10^0
13S	13S	WT	2.6×10^9	5.0×10^8	1.9×10^{-1}
13S.R2	13S	19K ⁻	1.2×10^9	6.0×10^8 *	5.0×10^{-1}
12S	12S	WT	1.4×10^9	4.1×10^4	2.9×10^{-5}
12S.R2	12S	19K ⁻	4.0×10^9	8.0×10^5 *	2.0×10^{-4}
12S.337	12S	19K ⁻	5.5×10^8	4.4×10^6 *	8.0×10^{-3}
9S	9S	WT	1.4×10^9	1.0×10^6	7.1×10^{-4}
9S.R2	9S	19K ⁻	8.1×10^8	1.0×10^4	1.2×10^{-5}

^a WT, Wild type.^b *, Large plaques.

be a connection between E1B 19K gene mutations stimulating E1A-dependent early expression and large-plaque formation.

Growth of mutant viruses in HeLa cells. Virus production by the mutant viruses was monitored in growth curves in HeLa cells, and the virus yield was determined by plaque assay on 293 cells. *dl309* and *dl337* produced large virus yields and displayed identical growth kinetics upon infection of HeLa cells (Fig. 7A). Neither virus was defective for growth in HeLa cells, indicating that functional E1B 19K protein is not essential for replication in HeLa cells of an otherwise wild-type virus, consistent with results previously reported (54, 78). On infection of HeLa cells with the 12S virus, the yield was considerably reduced, as expected, increasing only 30-fold from 24 to 96 h postinfection (Fig. 7B). Infection of HeLa cells with the 12S.337 virus caused considerably greater virus growth, yielding a 700-fold increase in virus production from 24 to 96 h postinfection (Fig. 7B). Therefore, in a 12S E1A viral background, the stimulation of viral gene expression that results from E1B 19K gene mutations conferred a greater virus replication capacity in HeLa cells.

E1B 19K gene mutations have the opposite effect on virus replication during infection of HeLa cells in the absence of

functional E1A products. A 9S E1A virus replicates poorly; however, a 9S virus with an E1B 19K gene point mutation does not replicate at all (Fig. 7C). Therefore, the E1B 19K protein is required for minimal virus replication in HeLa cells in the absence of E1A.

DISCUSSION

We have observed two apparently opposing effects of the E1B 19K tumor antigen on viral gene expression. E1B 19K protein functions to negatively regulate early and late gene expression in an E1A-dependent fashion during infection, but in the absence of E1A, a stimulatory effect of the E1B 19K protein on viral gene expression, DNA synthesis, and replication is unmasked. Both positive and negative effects of the E1B 19K gene on virus replication may explain the phenotypes of viruses with mutations in the E1B 19K gene.

E1A-dependent negative effect of the E1B 19K protein on viral gene expression. During the course of infection, the E1A proteins are primarily responsible for activating transcription of the early adenovirus promoters (Fig. 8A). Although the E1A proteins also possess the ability to repress enhancers, including the E1A enhancer (6, 21, 26, 27, 57, 74, 75), it is not known whether enhancer repression is a regulatory

FIG. 7. Growth curves of wild-type and mutant viruses in HeLa cells. Cells were infected with (A) *dl309* and *dl337*, at a multiplicity of infection of 1 PFU per cell; (B) 12S and 12S.337, at a multiplicity of infection of 0.1 PFU per cell; and (C) 9S and 9S.R2 at a multiplicity of infection of 0.1 PFU per cell. At the indicated times, crude cell lysates were made and the titers were determined by plaque assay on 293 cells.

FIG. 8. Model for E1B modulation of adenovirus early gene expression (see text for details). Abbreviation: wt, wild type.

mechanism for controlling adenovirus gene expression during productive infection. Deletion of the E1A enhancer causes a reduction of E1A mRNA levels at early times and has little effect at late times postinfection (25). Therefore, if repression of the E1A enhancer by the E1A proteins occurs during productive infection, either it has a relatively modest effect on adenovirus early gene expression, or more likely, repression is counteracted by stimulation of the enhancer.

Eliminating expression of the E1B 19K protein by infecting HeLa cells with an adenovirus mutant relieves the negative effect of E1B on viral gene expression, and there is an accelerated time course of early gene expression. The E1A proteins are overproduced, and, consequently, E1A-dependent viral processes such as transcription and virus replication are stimulated (Fig. 8B). The increased early gene expression in mutant-infected cells is then compounded by increased viral DNA replication, which, in turn, supplies more templates for transcription. For the 12S cDNA virus, which is deficient in activating the transcription of viral early genes, the increased viral gene expression induced by the loss of functional E1B 19K protein partially rescues this defective virus. This result also indicates that the 12S E1A protein is intrinsically capable of activating viral gene transcription, albeit to a limited extent. The ability of the 12S protein to activate transcription has varied and often depends on the particular assay involved (11, 36, 40, 56, 81). This may reflect differences in the amount or activity of the 12S protein produced or supplied under the different assay conditions. Another possibility is that the 12S E1A protein induces a cellular state that is conducive to viral gene expression. This function is probably the mechanism by which E1A activates cellular DNA synthesis and transcription of growth-related genes such as the PCNA gene (85). This property is common to both the 13S and 12S E1A proteins, but is absent in the 9S product.

The net result of E1B 19K gene mutations on gene expression is manifested by the *cyt*, *lp*, and *hr* phenotypes

during viral infection. The function of the E1B 19K protein in the down-regulation of viral gene transcription and replication may be an important requirement for correct temporal control and regulation of productive infection, thereby preventing premature death of the host cell before the virus life cycle is complete. One consequence of this regulation is that the E1B 19K protein influences the host cell range in which adenovirus can replicate (77, 79). E1B 19K gene mutations cause a virus to have a growth advantage during infection of less permissive human cells (WI38) (77). However, infection of HeLa or KB cells with the same mutant viruses results in *cyt*, *deg*, and *lp* phenotypes. A compromise in virus yield is evident in mutant-infected KB cells and is very probably caused by the severe cytopathic effects and premature death of the host cell (67, 68, 77).

Proteins that function to down-regulate gene expression and virus replication are not unique to adenovirus. Simian virus 40 large T antigen has an autoregulatory capability by repressing transcription of the viral early promoter at late times in infection by binding to specific DNA sites and turning off its own transcription (23, 51) and perhaps by interfering with gene activation by the cellular transcription protein AP2 (46). A factor that negatively regulates transcription in bovine papillomavirus has also been identified (39). The human immunodeficiency virus genome has been reported to encode a factor that down-regulates virus replication, although the mechanism of the down-regulation has not been determined (45). As mentioned above, adenovirus could potentially autoregulate gene expression by the E1A enhancer repression mechanism. Therefore, it is intriguing that there is an additional requirement for negative regulation by the E1B 19K protein; this may indicate either that the enhancer repression mechanism alone is not sufficient to modulate E1A levels during productive infection or that E1B plays a role in modulating E1A-dependent enhancer repression.

E1A-independent stimulatory effect of E1B 19K protein on viral gene transcription. In contrast to the regulation described above, in the absence of functional E1A proteins and E1A-dependent transactivation, the E1B 19K protein stimulates adenovirus gene transcription to a small degree during infection (Fig. 8C). An E1B 19K gene point mutation eliminates the stimulatory activity and causes diminished transcription of adenovirus early genes and virus replication (Fig. 8D). The vast majority of this effect is indirect and is merely the result of an increase in the viral template copy number owing to increased viral DNA synthesis. The mechanism by which the E1B 19K protein stimulates viral DNA replication in the absence of E1A is not yet known. It is likely that the same phenomenon occurs in the presence of E1A, but virus replication is so efficient under these conditions that it may be difficult to observe. This phenomenon may be further masked by E1B down-regulating E1A activation of transcription. One function of the E1B 19K protein, which may be related to the increased viral DNA synthesis, is its ability to protect or stabilize DNA. Expression of the E1B 19K protein in 9S virus-infected cells might stabilize viral DNA templates by preventing their degradation and thereby promote viral DNA synthesis.

Possible mechanisms by which the E1B 19K protein controls gene expression. It has been suggested that the E1B region stimulates expression of the E1A proteins, since cotransfection of E1A and E1B genes results in higher E1A levels than transfection of E1A alone in transient assays (61) and in stable transformants (72). This may be due to a *cis*-acting element within E1B gene and not to the action of the proteins

(31). A small stimulatory activity, specifically attributed to the E1B 19K protein, on expression from adenovirus promoters has been observed in transient-transfection assays (27a, 52, 83; E. White, unpublished results). In addition to the adenovirus promoters, the E1B 19K protein is capable of stimulating expression of the cellular hsp70 promoter (27a) and other viral promoters (83; White, unpublished). The 19K protein therefore appears to be a general, but weak, stimulator of gene expression in transient-transfection assays. This small stimulatory activity of the 19K protein may also account for the small increase in DBP expression in 9S-infected cells in the presence of hydroxyurea. However, the increases in expression of transfected genes could be indirectly and solely due to stabilization of the transfected DNA by the 19K protein. For the 9S virus, at relatively low copy number, the largest effect of the stabilization of viral DNA might be to promote viral DNA synthesis. For transfected DNA, at relatively high copy number, higher levels of gene expression could result from an extended half-life of the DNA template. Results obtained by Alwine (1) have shown that transfected DNA ordinarily has a short half-life in transfected cells; however, in 293 cells which express E1A and E1B proteins, the transfected DNA is much more stable. Perhaps the E1B 19K protein protects transfected plasmid DNAs in the same way as it prevents degradation of chromosomal DNA in infected cells. To determine whether the 19K protein directly stimulates gene expression or acts indirectly by affecting DNA template levels, template levels and gene expression (transcription, mRNA levels, and protein synthesis) in both transfected and infected cells must be monitored.

The stimulatory effect of the E1B 19K protein in the absence of E1A during infection and in transient assays may be mechanistically related to its negative effect on viral gene expression during infection in the presence of E1A. That is, stabilization or compartmentalization of DNA in the nucleus may impede E1A transcriptional activation, but may promote viral DNA replication under adverse conditions when the E1A proteins are absent. The means by which E1B could interfere with E1A function could include a direct mechanism, such as altering the activity of DBPs that are required for transcription of adenovirus genes, or an indirect mechanism, such as an effect on the cell cycle, nuclease activity, chromatin structure, or nuclear structure.

The negative effect of E1B on viral gene expression may reflect modulation of the ability of the E1A proteins to repress enhancers in the presence of E1B. Increased repression of the E1A enhancer in the presence of E1B, for example, could down-regulate adenovirus gene expression during viral infection. This may be especially important at late times during infection, when E1A levels are down-regulated relative to the increase in viral DNA replication and late gene transcription. It is interesting that overexpression of E1A and the DBP in E1B mutant-infected cells occurs after viral DNA replication, which is also the time of maximal expression of the E1B 19K protein in cells infected with the wild-type virus (Fig. 2). We are currently measuring the ability of the E1B mutant viruses to repress the simian virus 40 and E1A enhancers during productive infection, in order to address this question directly. Preliminary data indicate that the activity of the simian virus 40 enhancer, when carried on an adenovirus chromosome, is either slightly repressed or unaffected by coinfection with the 12S virus. On coinfection with the 12S.337 virus, however, the simian virus 40 enhancer activity is stimulated. This is similar to results reported here for expression of adenovirus

genes. That is, eliminating E1B 19K gene function either increases the ability of the 12S E1A protein to activate transcription or prevents its ability to repress transcription.

During a wild-type adenovirus infection, in the presence of the E1B 19K protein the activity of the E1A proteins is altered, resulting in reduced early gene transcription. This indicates that there is a functional interaction between the E1A and E1B 19K proteins. The mechanism by which the E1A proteins stimulate and repress transcription, or how the E1B 19K protein may alter their activity, is not yet known. Recent data suggest that the E1A proteins alter the activity of cellular transcription factors (41). It may be that the increased levels of the E1A proteins in E1B mutant-infected cells are sufficient to cause derepression of the E1A enhancer. In that case, the E1B 19K protein could potentially decrease the stability of the E1A proteins or mRNAs or their transport to and from the nucleus. The 19K protein is known to be associated with the nuclear envelope of infected cells (76), where it might regulate nuclear protein or RNA transport. We believe that it is unlikely that the role of the E1B 19K protein in regulating gene expression is solely due to modulation of E1A levels during infection. Synthesis of high levels of the E1A proteins, by infection with the wild-type virus in the presence of hydroxyurea or by using high multiplicities of infection, does not result in the appearance of the E1B mutant phenotypes (78; White, unpublished). Therefore, induction of higher E1A levels during infection is not itself sufficient to induce the E1B mutant phenotypes; this suggests that it may be the activity, and not the amount, of the E1A proteins that is altered. Finally, the E1A proteins are known to be posttranslationally modified by phosphorylation (15, 24, 82). Conceivably, alterations in the phosphorylation state of the E1A proteins by the E1B 19K protein may reflect changes in transcriptional regulation.

We have previously shown that induction of the E1B mutant phenotypes is dependent on the expression of the E1A proteins (79). Recently, by constructing E1A-E1B double-mutant viruses, we have mapped the induction of the DNA degradation phenotype to E1A domain 1 (E. White, manuscript in preparation). These data suggest that the ability of the E1A proteins to stimulate cellular DNA synthesis, which is encoded by conserved domain 1 in the E1A protein, may be related to induction of the DNA degradation phenotype. Identification of the E1A domain(s) required for the negative effect of E1B on gene expression may shed some light on the functional mechanism of repression of early gene expression by E1B. We have demonstrated here that E1B 19K gene mutations in an E1A 12S cDNA virus display this negative effect, indicating that the 13S unique region is not required. Further genetic analysis should elucidate which E1A domain functions are required for repression of early gene transcription by E1B.

ACKNOWLEDGMENT

This work was supported by Public Health Service grant CA13106 from the National Cancer Institute.

LITERATURE CITED

1. Alwine, A. C. 1985. Transient gene expression control: effects of transfected DNA stability and *trans*-activation by viral early proteins. *Mol. Cell. Biol.* 5:1034-1042.
2. 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.
3. 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.
4. Berk, A. J. 1986. Adenovirus promoters and E1A transactivation. *Annu. Rev. Genet.* 20:45-79.
 5. Bernards, R., M. G. W. deLeeuw, A. Houweling, and A. J. van der Eb. 1986. Role of the adenovirus early region 1B tumor antigens in transformation and lytic infection. *Virology* 150:126-139.
 6. Borrelli, E., R. Hen, and P. Chambon. 1984. Adenovirus-2 E1A products repress enhancer-induced stimulation of transcription. *Nature (London)* 312:608-612.
 7. Branton, P. E., S. T. Bayley, and F. L. Graham. 1985. Transformation by human adenoviruses. *Biochim. Biophys. Acta* 780: 67-94.
 8. Chinnadurai, G. 1983. Adenovirus 2 $1p^+$ locus codes for a 19kd tumor antigen that plays an essential role in cell transformation. *Cell* 33:759-766.
 9. D'Halluin, J. C., C. Allart, C. Cousin, P. A. Boulanger, and G. Martin. 1979. Adenovirus early function required for the protection of viral and cellular DNA. *J. Virol.* 32:61-71.
 10. Enzo, H., R. B. Lai Fatt, and S. Mak. 1981. Degradation of intracellular DNA in KB cells infected with *cyr* mutants of human adenovirus type 12. *J. Virol.* 40:20-27.
 11. Ferguson, B., N. Jones, J. Richler, and M. Rosenberg. 1984. Adenovirus E1a gene product expressed at high level in *Escherichia coli* is functional. *Science* 224:1343-1346.
 12. Flint, S. J. 1984. Cellular transformation by adenovirus. *Pharmac. Ther.* 26:59-88.
 13. Fukui, Y., I. Saito, K. Shiroki, and H. Shimojo. 1984. Isolation of transformation-defective, replication-nondefective early region 1B mutants of adenovirus 12. *J. Virol.* 49:154-161.
 14. Gaynor, R. B., D. Hillman, and A. J. Berk. 1984. Adenovirus early region 1A protein activates transcription of a nonviral gene introduced into mammalian cell by infection or transfection. *Proc. Natl. Acad. Sci. USA* 81:1193-1197.
 15. Gaynor, R. B., A. Tsukamoto, C. Montell, and A. J. Berk. 1982. Enhanced expression of adenovirus transforming proteins. *J. Virol.* 44:276-285.
 16. Graham, F. L., P. J. Abrahams, C. Mulder, H. L. Heijnecker, S. O. Warnaar, F. A. J. deVries, and A. J. van der Eb. 1974. Studies of *in vitro* transformation by DNA and DNA fragments of human adenovirus and simian virus 40. Cold Spring Harbor Symp. Quant. Biol. 39:637-650.
 17. Graham, F. L., T. Harrison, and J. Williams. 1978. Defective transforming capacity of adenovirus type 5 host-range mutants. *Virology* 86:10-21.
 18. Graham, F. L., J. Smiley, W. C. Russell, and R. Nairn. 1977. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J. Gen. Virol.* 36:59-77.
 19. Greenberg, M. E., and E. B. Ziff. 1984. Stimulation of 3T3 cells induces transcription of the *c-fos* proto-oncogene. *Nature (London)* 311:433-438.
 20. Groudine, M., M. Peretz, and H. Weintraub. 1981. Transcriptional regulation of hemoglobin switching in chicken embryos. *Mol. Cell. Biol.* 1:281-288.
 21. Guilfoyle, R. A., W. P. Osheroff, and M. Rossini. 1985. Two functions encoded by adenovirus early region 1A are responsible for the activation and repression of the DNA-binding protein gene. *EMBO J.* 4:707-713.
 22. Haley, K. P., J. Overhauser, 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.
 23. Hansen, U., D. G. Tenen, D. M. Livingston, and P. A. Sharp. 1981. T antigen repression of the SV40 early transcription from two promoters. *Cell* 27:603-612.
 24. Harlow, E., B. Franza, Jr., and C. Schley. 1985. Monoclonal antibodies specific for adenovirus early region 1A proteins: extensive heterogeneity in early region 1A products. *J. Virol.* 55:533-546.
 25. Hearing, P., and T. Shenk. 1983. The adenovirus type 5 E1A transcriptional control region contains a duplicated enhancer element. *Cell* 33:695-703.
 26. 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.
 27. Hen, R., E. Borrelli, C. Fromental, P. Sassone-Corsi, and P. Chambon. 1986. A mutated polyoma virus enhancer which is active in undifferentiated embryonal carcinoma cells is not repressed by adenovirus-1 E1A products. *Nature (London)* 321: 249-250.
 - 27a. Herrmann, C. H., C. V. Déry, and M. B. Mathews. 1987. Transactivation of host and viral genes by the adenovirus E1B 19K tumor antigen. *Oncogene* 2:25-35.
 28. Hirt, B. 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. *J. Mol. Biol.* 26:365.
 29. Houweling, A., P. J. van den Elsen, and A. J. van der Eb. 1980. Partial transformation of primary rat cells by the leftmost 4.5% fragment of adenovirus 5 DNA. *Virology* 105:537-550.
 30. Imperiale, M. J., H. Kao, L. T. Feldman, J. R. Nevins, and S. Strickland. 1984. Common control of the heat shock gene and early adenovirus genes: evidence for a cellular E1A-like activity. *Mol. Cell. Biol.* 4:867-874.
 31. Jochimsen, A. B., L. T. C. Peltenburg, M. F. W. te Pas, C. M. de Wit, J. L. Bos, and A. J. van der Eb. 1987. Activation of adenovirus 5 E1A transcription by region E1B in transformed primary rat cells. *EMBO J.* 6:3399-3405.
 32. 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.
 33. Jones, N., and T. Shenk. 1979. Isolation of adenovirus type 5 host range deletion mutants defective for transformation of rat embryo cells. *Cell* 17:683-689.
 34. Kaczmarek, L., B. Ferguson, M. Rosenberg, and R. Baserga. 1986. Induction of cellular DNA synthesis by purified adenovirus E1A proteins. *Virology* 152:1-10.
 35. Kao, H., and J. R. Nevins. 1983. Transcriptional activation and subsequent control of the human heat shock gene during adenovirus infection. *Mol. Cell. Biol.* 3:2058-2065.
 36. Kripl, B., B. Ferguson, N. Jones, M. Rosenberg, and H. Westphal. 1985. Mapping of functional domains in adenovirus E1A proteins. *Proc. Natl. Acad. Sci. USA* 82:7480-7484.
 37. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680-685.
 38. Lai Fatt, R. B. and S. Mak. 1982. Mapping of an adenovirus function involved in the inhibition of DNA degradation. *J. Virol.* 42:969-977.
 39. Lambert, P. F., B. A. Spalholz, and P. M. Howley. 1987. A transcriptional repressor encoded by BPV-1 shares a common carboxy-terminal domain with the E2 transactivator. *Cell* 50:69-78.
 40. Leff, T., R. Elkaim, C. R. Goding, P. Jalinot, P. Sassone-Corsi, M. Perriacaudet, C. Kedinger, and P. Chambon. 1984. Individual products of the adenovirus 12S and 13S E1A mRNAs stimulate viral E1I and E1II expression at the transcriptional level. *Proc. Natl. Acad. Sci. USA* 81:4381-4385.
 41. Leong, K., and A. J. Berk. 1986. Adenovirus early region 1A protein increases the number of template molecules transcribed in cell-free extracts. *Proc. Natl. Acad. Sci. USA* 83:5844-5848.
 42. Lewis, J. B., and C. W. Anderson. 1987. Identification of adenovirus type 2 early region 1B proteins that share the same amino terminus as do the 495R and 155R proteins. *J. Virol.* 61: 3877-3888.
 43. 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.
 44. 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.
 45. Luciw, P. A., C. Cheng-Mayer, and J. A. Lewy. 1987. Mutational analysis of the human immunodeficiency virus: the *orf-B* region down-regulates virus replication. *Proc. Natl. Acad. Sci. USA* 84:1434-1438.
 46. Mitchell, P. J., C. Wang, and R. Tjian. 1987. Positive and

- negative regulation of transcription *in vitro*: enhancer-binding protein AP-2 is inhibited by SV40 T antigen. *Cell* 50:847-861.
47. Montell, C., G. Courtois, C. Eng, and A. Berk. 1984. Complete transformation by adenovirus 2 requires both E1A proteins. *Cell* 36:951-961.
 48. 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.
 49. Moran, E., and M. B. Mathews. 1987. Multiple functional domains in the adenovirus E1A gene. *Cell* 48:177-178.
 50. Moran, E., B. Zerler, T. M. Harrison, and M. B. Mathews. 1986. Identification of separate domains in the adenovirus E1A gene for immortalization activity and the activation of virus early genes. *Mol. Cell. Biol.* 6:3470-3480.
 51. Myers, R. M., D. C. Rio, A. K. Robbins, and R. Tjian. 1981. SV40 gene expression is modulated by the cooperative binding of T antigen to DNA. *Cell* 25:373-384.
 52. Natarajan, V. 1986. Adenovirus-2 E1a and E1b gene products regulate enhancer mediated transcription. *Nucleic Acids Res.* 14:9445-9455.
 53. Pilder, S., K. Leppard, J. Logan, and T. Shenk. 1986. Functional analysis of the adenovirus E1B 55K polypeptide. *Cancer Cells* 4:285-290.
 54. 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.
 55. Pilder, S., M. Moore, J. Logan, and T. Shenk. 1986. The adenovirus E1B 55K transforming polypeptide modulates transport or cytoplasmic stabilization of viral and host cell mRNAs. *Mol. Cell. Biol.* 6:470-476.
 56. Richter, J. D., P. Young, N. C. Jones, B. Krippl, M. Rosenberg, and B. Ferguson. 1985. A first exon-encoded domain of E1A sufficient for posttranslational modification, nuclear-localization, and induction of adenovirus E3 promoter expression in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* 82:8434-8438.
 57. Rossini, M. 1983. The role of adenovirus early region 1A in the regulation of early regions 2A and 1B expression. *Virology* 131:49-58.
 58. Ruley, H. E. 1983. Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture. *Nature (London)* 304:602-606.
 59. Sassone-Corsi, P., and E. Borrelli. 1987. Promoter trans-activation of protooncogenes *c-fos* and *c-myc*, but not *c-Ha-ras*, by products of adenovirus early region 1A. *Proc. Natl. Acad. Sci. USA* 84:6430-6433.
 60. Schneider, J. F., F. Fisher, C. R. Goding, and N. C. Jones. 1987. Mutational analysis of the adenovirus E1a gene: the role of transcriptional regulation in transformation. *EMBO J.* 6:2053-2060.
 61. 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.
 62. Spindler, K. R., C. Y. Eng, and A. Y. Berk. 1985. An adenovirus early region 1A protein is required for maximal viral DNA replication in growth-arrested human cells. *J. Virol.* 53:742-750.
 63. Stabel, S., P. Argos, and L. Philipson. 1985. The release of growth arrest by microinjection of adenovirus E1A DNA. *EMBO J.* 4:2329-2336.
 64. Stein, R., and E. B. Ziff. 1984. HeLa cell B-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.
 65. Stephens, C., and E. Harlow. 1987. Differential splicing yields novel adenovirus 5 E1A mRNAs that encode 30 kd and 35 kd proteins. *EMBO J.* 6:2027-2035.
 66. Stillman, B. 1986. Functions of the adenovirus E1B tumour antigens. *Cancer Surv.* 5:389-404.
 67. Subramanian, T., M. Kuppuswamy, J. Gysbers, S. Mak, and G. Chinnadurai. 1984. 19-kDa tumor antigen coded by early region E1b of adenovirus 2 is required for efficient synthesis and for protection of viral DNA. *J. Biol. Chem.* 259:11777-11783.
 68. Subramanian, T., M. Kuppuswamy, S. Mak, and G. Chinnadurai. 1984. Adenovirus *cyt*⁺ locus, which controls cell transformation and tumorigenicity, is an allele of *lp*⁺ locus, which codes for a 19-kilodalton tumor antigen. *J. Virol.* 52:336-343.
 69. Takemori, N., C. Cladaras, B. Bhat, A. J. Conley, and W. S. M. Wold. 1984. *cyt* gene of adenoviruses 2 and 5 is an oncogene for transforming function in early region E1B and encodes the E1B 19,000-molecular-weight polypeptide. *J. Virol.* 52:793-805.
 70. Takemori, N., J. L. Riggs, and C. Aldrich. 1968. Genetic studies with tumorigenic adenoviruses. 1. Isolation of cytotoxic (cyt) mutants of adenovirus type 12. *Virology* 36:575-586.
 71. Ulfendahl, P. J., S. Linder, J.-P. Kreivi, K. Nordqvist, C. Svensson, H. Hultberg, and G. Akusjärvi. 1987. A novel adenovirus-2 E1A mRNA encoding a protein with transcription activation properties. *EMBO J.* 6:2037-2044.
 72. van den Elsen, P., S. de Pater, A. Houweling, and A. J. van der Eb. 1983. Expression of region E1b of human adenovirus in the absence of E1A is not sufficient for complete transformation. *Virology* 128:377-390.
 73. van der Eb, A. J., C. Mulder, F. L. Graham, and A. Houweling. 1977. Transformation with specific fragments of adenovirus DNAs. I. Isolation of specific fragments with transforming activity of adenovirus 2 and 5 DNA. *Gene* 2:115-132.
 74. Velcich, A., F. G. Kern, C. Basilico, and E. B. Ziff. 1986. Adenovirus E1a proteins repress expression from polyomavirus early and late promoters. *Mol. Cell. Biol.* 6:4019-4025.
 75. Velcich, A., and E. Ziff. 1985. Adenovirus E1a proteins repress transcription from the SV40 early promoter. *Cell* 40:705-716.
 76. White, E., S. H. Blose, and B. W. Stillman. 1984. Nuclear envelope localization of an adenovirus tumor antigen maintains the integrity of cellular DNA. *Mol. Cell. Biol.* 4:2865-2875.
 77. White, E., B. Faha, and B. Stillman. 1986. Regulation of adenovirus gene expression in human WI38 cells by an E1B-encoded tumor antigen. *Mol. Cell. Biol.* 6:3763-3773.
 78. White, E., T. Grodzicker, and B. W. Stillman. 1984. Mutations in the gene encoding the adenovirus E1B 19K tumor antigen cause degradation of chromosomal DNA. *J. Virol.* 52:410-419.
 79. White, E., and B. Stillman. 1987. Expression of adenovirus E1B mutant phenotypes is dependent on the host cell and on synthesis of E1A proteins. *J. Virol.* 61:426-435.
 80. Williams, J., B. D. Karger, Y. S. Ho, C. L. Castiglia, T. Mann, and S. J. Flint. 1986. The adenovirus E1B 495R protein plays a role in regulating the transport and stability of the viral late messages. *Cancer Cells* 4:275-284.
 81. Winberg, G., and T. Shenk. 1984. Dissection of overlapping functions within the adenovirus type 5 E1a gene. *EMBO J.* 3:1907-1912.
 82. Yee, S.-P., D. T. Rowe, M. L. Tremblay, M. McDermott, and P. E. Branton. 1983. Identification of human adenovirus early region 1 products by using antisera against synthetic peptides corresponding to the predicted carboxy termini. *J. Virol.* 46:1003-1013.
 83. Yoshida, K., L. Venkatesh, M. Kuppuswamy, and C. Chinnadurai. 1987. Adenovirus transforming 19-kD T antigen has an enhancer-dependent *trans*-activation function and relieves enhancer repression by viral and cellular genes. *Genes Dev.* 1:645-658.
 84. 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.
 85. Zerler, B., R. J. Roberts, M. B. Mathews, and E. Moran. 1987. Different functional domains of the adenovirus E1A gene are involved in regulation of host cell cycle products. *Mol. Cell. Biol.* 7:821-829.