MOLECULAR AND CELLULAR BIOLOGY, Nov. 1986, p. 3798–3806
0270-7306/86/113798-09$02.00/0

Vol. 6, No. 11

Cellular Promoters Incorporated into the Adenovirus Genome: Effects of Viral Regulatory Elements on Transcription Rates and Cell Specificity of Albumin and β-Globin Promoters

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Received 24 March 1986/Accepted 3 July 1986

In the accompanying paper (Friedman et al., Mol. Cell. Biol. 6:3791–3797, 1986), hepatoma-specific expression of the rat albumin promoter within the adenovirus genome was demonstrated. However, the rate of transcription was very low compared with that of the endogenous chromosomal albumin gene. Here we show that in hepatoma cells the adenovirus E1A enhancer, especially in the presence of E1A protein, greatly stimulates transcription from the albumin promoter but not the mouse β-globin promoter. This enhancer-dependent stimulation did not occur in myeloma cells in which a virus containing a immunoglobulin promoter and enhancer did function. These experiments suggest a limited distribution in cultured differentiated cells of cell-specific transcription factors. However, either the regulation of such cell-specific factors breaks down in other cultured cells, or strictly cell-specific factors are not at play in controlling cell-specific transcription, because HeLa cells could transcribe the albumin promoter from the same start site about 10% as well as hepatomas could and 293 cells could transcribe both albumin and globin promoters.

Cell-specific expression from the albumin promoter within the adenovirus genome occurs at a low rate compared with that of the endogenous gene in human hepatoma cells or the mouse albumin gene in primary hepatocytes, as was reported in the previous paper (10). To determine whether this low rate of transcription could be increased while maintaining cell-specific transcription we added the adenovirus E1A enhancer sequences (18) to a set of new viruses and provided the E1A proteins during infection of both differentiated and undifferentiated cells.

E1A proteins are known to have positive transcriptional effects on different viral and cellular genes in the following cases: early viral genes on the viral genome, including the E1A gene itself (3, 19, 22, 30, 31); early viral genes on cellular chromosomes (8); activation of the β-globin gene on a transfected circular plasmid (16, 34); and activation of chromosomal heat shock (23) and β-tubulin genes (33). In addition, E1A proteins can cause a suppressing effect on transfected enhancer-dependent transcriptional units in the circular simian virus 40 genome (4, 35).

In this paper we show that the adenovirus type 5 E1A enhancer sequences on a viral genome will stimulate the albumin promoter on a viral genome about fivefold when the virus infects human hepatoma cells. The presence of E1A proteins (provided by a helper virus) further increases transcription by another factor of 10 to 20, so that transcription from the viral chromosomes (measured directly) exceeds that of the endogenous hepatoma cell genes. In a similar virus construction, globin transcription could not be detected in hepatoma cells. Moreover, albumin expression from the viral albumin promoter could not be detected in myeloma (MPC11) cells. These results suggest that cell-specific enhancers, known to exist in lymphocytes (2, 9, 12, 32, 36), might also exist to raise albumin transcription to a high rate in hepatocytes.

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In contrast to the apparent cell specificity observed in differentiated cell lines, about 2 to 10% as much expression of globin and albumin gene-driven transcription occurred in undifferentiated human cells (HeLa or 293 cells) in the simultaneous presence of both the viral E1A protein and its enhancer element. We discuss the implications of these experiments for the nature of transcriptional factors, the genomic sequences which might affect both specificity and rate, and the mode action of viral stimulatory factors.

MATERIALS AND METHODS

Cells and virus. Monolayer cultures of human HeLa, HepG2, and 293 cells and suspension cultures of mouse MPC11 cells were grown or maintained in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum. All virus stocks were propagated and titered by plaque quantitation on 293 cell monolayer cultures. H5dl313 (*dl*313) was provided by T. Shenk (22).

Plasmid and recombinant virus construction. The plasmid pAdBgl6 was modified to contain adenovirus sequences extending from the left end of the viral genome to nucleotide 454 (*Pvu*II enzyme site) and thus still lacking the E1A and E1B region up to 3320 base pairs. A *Bgl*II linker was added to the *Pvu*II-generated 3′ DNA terminus at position 454 so as to introduce segments of various genes (rat albumin and mouse β-major globin) downstream from the E1A enhancer sequences and upstream of the E1B gene region. These plasmids were then used to introduce both the viral and cellular sequences into the genome of H5In340 virus as described in the accompanying paper (10).

RESULTS

Virus constructions and RNA assays. The viruses used in this work are diagrammed in Fig. 1 and include the 194 series described in the accompanying paper (10) plus Alb454 and Glo454, which contain the E1A enhancer region (18) within

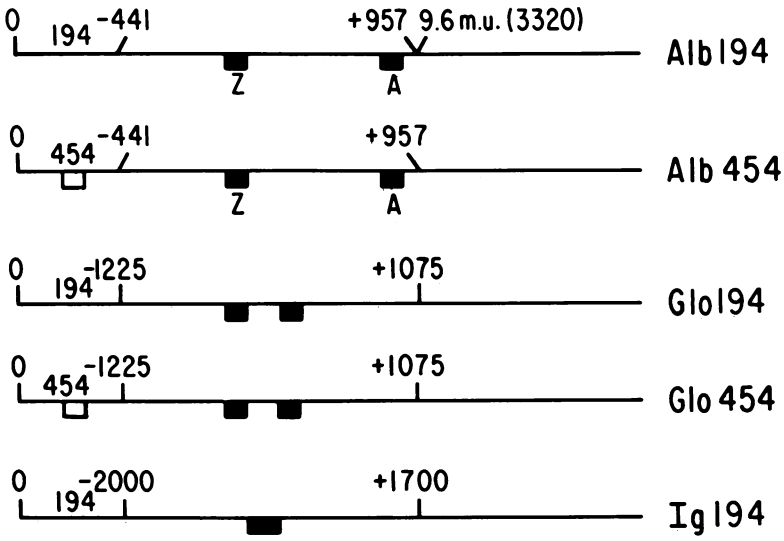

FIG. 1. Genomic maps of recombinant adenoviruses. Adenovirus recombinants were isolated by overlap recombination in 293 cells. The preparation of the 194 series was described previously (10). Albumin and globin sequences were also introduced into similar virus constructions that now include 454 nucleotides from the left end of the viral genome. This series of viruses (Alb454 and Glo454) contained the E1A enhancer and DNA-packaging elements (18). The filled-in boxes represent exon regions, and open boxes represent the E1A enhancer and DNA-packaging elements. The + and − symbols represent nucleotide distances relative to the known transcriptional start sites for the three cellular promoter regions. m.u., Map units.

the 454 nucleotides of the adenovirus left end. Infection of cells with these viruses produces mRNAs that can be scored with labeled sp6-generated RNA probes as described in the first paper (10). The production of recombinant E1B-containing mRNAs thus allows a test of promoter function in the 194 and 454 series of viruses (Fig. 2). In addition the nonreplicating viruses can be complemented with *dl*313, which lacks the E1B promoter and most of the E1B transcription unit. The coinfecting virus produced E1A protein from an mRNA that had only 147 nucleotides of E1B sequences at its 3′ end. This mRNA therefore protected only 147 nucleotides of the sp6 probe (Fig. 2).

With the new set of viruses containing viral enhancers and a technique for delivering E1A protein during virus infection without interfering with the ability to score albumin or globin promoter-driven E1B transcription, we tested the effects of viral regulatory elements under a variety of circumstances.

E1A enhancer, especially in presence of E1A proteins, boosts the rate of the viral albumin promoter activity in HepG2 cells. The effect of viral regulatory elements (enhancer and E1A protein) on the albumin and globin promoters in HepG2 cells was tested first. Infections with Alb194, Alb454, Glo194, or Glo454 virus alone or together with the *dl*313 virus were allowed to proceed for only 7 h so that no viral DNA replication occurred. Total cell RNA was isolated, and equal amounts of RNA from each infection were assayed by the sp6 technique for the presence of E1B-containing RNA.

HepG2 cells infected with Alb194 or Alb194 and *dl*313 contained small and about equal amounts of E1B mRNA (Fig. 2, first two lanes), indicating no stimulus by the E1A protein alone. After 7 h of infection, this RNA was about one-fifth as abundant as after a 24-h infection of the HepG2 cells that was described in the accompanying paper (10).

However, the presence of the viral enhancer sequences in the Alb454 virus resulted in an approximately fivefold increase in the accumulation of the E1B mRNAs over infections with the Alb194 virus (Fig. 2, lane 7; note the fivefold-longer autoradiographic exposure for lanes 1 and 2). Furthermore, when the E1A proteins were present together with the enhancer (*dl*313 and Alb194, coinfection), at least 100-fold more mRNA was accumulated than with the Alb194 virus infection (Fig. 2, lanes 1 and 8). In contrast to the results with the albumin promoter, HepG2 cells infected with the Glo194 or Glo454 virus either with or without E1A protein still did not produce any E1B RNA signal (Fig. 2, lanes 3 through 6). Thus, in hepatoma cells the E1A protein together with the E1A enhancer positively affected gene expression only if at least a low level of expression was already operating. Replication of the Alb454 virus apparently did not occur in any of these infections and simply increase the possible template number because no protein IX mRNA could be detected (see absence of 205-nucleotide band in lanes 7 and 8).

The increased production of mRNA brought about by the viral enhancer and the E1A protein did not change the initiation site used in the rat albumin DNA. The same 5′-end assay described earlier (Fig. 3 in reference 10, also see Fig. 5) showed that the same start site was used. Moreover, with the 5′-end assays, a quantitative comparison of steady-state RNA was possible. Cells infected for only 7 h with Alb454 and the helper virus contained about 25% as much RNA as rat hepatoma H4II cells (data not shown). Thus, albumin RNA production in the fully enhanced state from the virus genome was in the same range as in hepatoma cells.

Albumin promoter containing the E1A enhancer does not function in MPC11 cells regardless of E1A proteins. A possible explanation for the results of HepG2 cells is that the albumin promoter on the viral genome can be nonspecifically activated in all cells by the E1A enhancer and the E1A protein while the globin promoter in the virus cannot. To test this, MPC11 cells, a mouse myeloma cell line, were infected with the Alb454 or Ig194 virus with or without *dl*313 for a 24-h period, and whole-cell RNA was assayed by the sp6

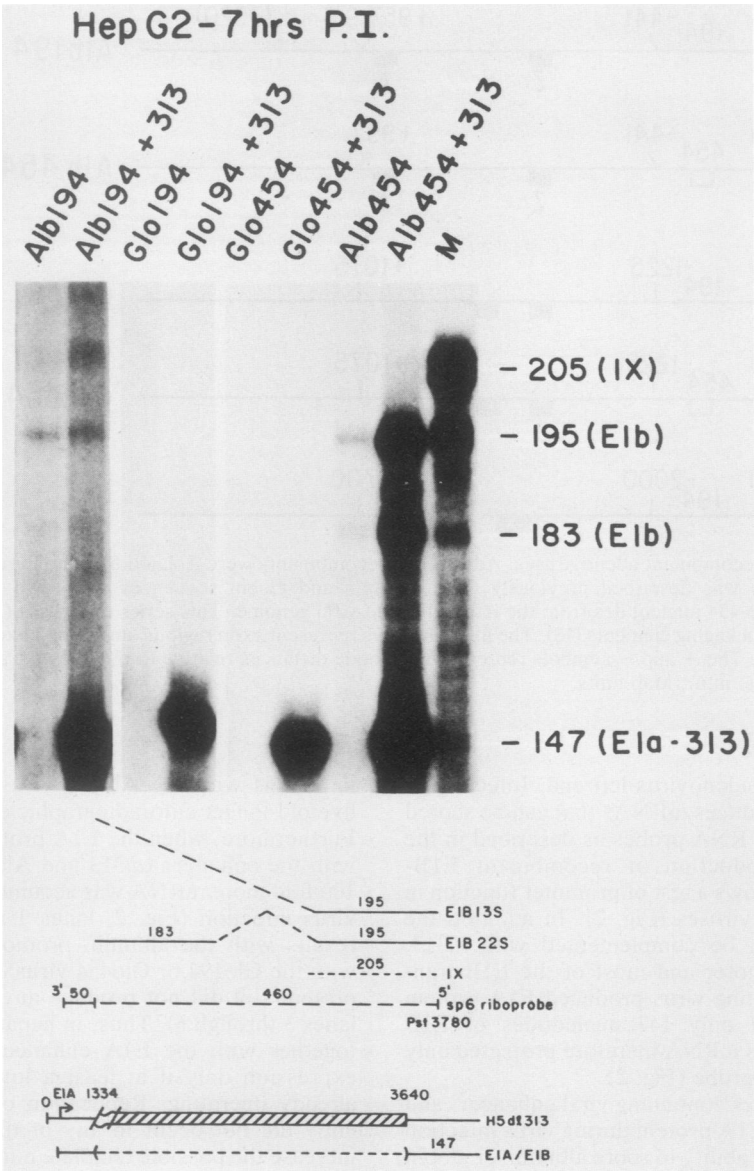

FIG. 2. Analysis of E1B mRNA species present at 7 h postinfection (P.I.) of HepG2 cells with recombinant adenoviruses. Total cytoplasmic RNA was isolated from HepG2 cells infected with 20 PFU of the indicated virus per cell, and equal quantitites (15 μg) were assayed for the presence of E1B exon RNA (see Fig. 1 and text). The autoradiographic exposure was 1 day for all of the lanes except the Alb194 and Alb194 + *dl*313 lanes, which were exposed for 5 days. The full-length labeled sp6 RNA probe of 510 nucleotides is protected over various lengths by different mRNAs: mRNAs spliced like 22S E1B mRNA protect 195 and 183 nucleotides; those spliced like 13S mRNAs protect 195 nucleotides; mRNA from *dl*313 protects 147 nucleotides, and protein IX RNA protects 205 nucleotides. The dashed lines that depict the E1B 13S and 22S mRNAs represent intronic sequences that are spliced out of the primary transcript. Deleted viral DNA sequences are depicted by a hatched box. The sp6 riboprobe contains adenovirus DNA sequences which span the *Bgl*II (nucleotide 3320) to the *Pst*I (nucleotide 3760) enzyme sites.

antisense-labeled RNA assay (Fig. 3). The Alb454 and Glo454 viruses did not generate E1B RNA even in the presence of E1A protein, while the Ig194 virus did. This was true even though the infection was allowed to go on for 24 h, since viral DNA replication does not occur in mouse cells. Possibly because the DNA in the Ig194 virus contained a cellular enhancer element, the rate of expression of E1B RNA was significantly greater in cells infected with Ig194 than that generated by the Alb194 virus in HepG2 cells at 24 h postinfection (compare Fig. 3, lanes 1 and 2, with Fig. 2, lanes 1 and 2). Furthermore, the presence of E1A protein did not appear to suppress the expression of the immunoglobulin promoter. However, the lack of activity in mouse myeloma cells of the Alb454 virus (containing the viral enhancer) even in the presence of E1A proteins reinforced the possibility that without tissue-specific transcriptional activation of the albumin promoter, the viral regulatory elements did not boost transcription. The failure of the Alb454 virus to express E1B mRNAs in mouse myeloma cells was not due to a species barrier, since the rat albumin promoter was shown earlier to be active in mouse hepatocytes (10).

Direct measurements of transcription from the viral albumin promoters in Alb454 virus. Since the accumulation in HepG2 cells of the E1B mRNAs originating from the albu-

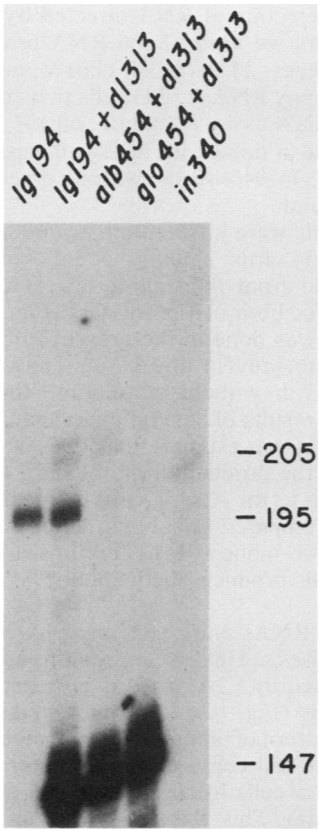

FIG. 3. Analysis of E1B mRNA species present at 24 h after infection of MPC11 cells with recombinant adenoviruses. Following infection for 24 h with the indicated virus, total-cell RNA was isolated, and the sp6 assay for E1B mRNA was carried out. RNA (15 μg) was assayed, and autoradiographs were exposed for 1 day. Sizes (in nucleotides) are indicated.

min promoter was considerably increased by the dual presence of E1A proteins and the E1A enhancer, we attempted to measure the rate of transcription from the reconstructed albumin E1B gene in HepG2 nuclei. Nuclei from HepG2 cells coinfected with 20 PFU of *dl*313 and Alb454 or Glo454 or with 20 PFU of In340 virus per cell were isolated after 7 h of infection. Similarly, nuclei were isolated from HepG2 cells infected for 24 h with Alb454 or Glo454 virus alone. Labeled nascent RNA chains were prepared and hybridized to DNA blots affixed to nitrocellulose filters, and RNase-resistant hybrids were scored.

In the sample taken at 7 h after infection, no specific transcriptional signal from cells infected with either the Alb454 of Glo454 virus was detected by hybridization to a 365-nucleotide E1B region DNA fragment (dot labeled E1B, Fig. 4, top row). However, we were able to detect a signal above background with a DNA fragment containing 957 bases from within the rat albumin gene (dot labeled r.alb, Fig. 4). Although the rat albumin gene and human albumin gene sequences diverge within this segment of DNA, a slight hybridization signal from the human albumin gene probably still occurs (see r.alb dot compared with pBR in panel of Glo454- and *dl*313-infected cells and In340-infected cells). However, the RNA transcribed from the human albumin gene in HepG2 cells did not prevent detection of the transcriptional signal from the rat albumin sequences resident within the Alb454 virus (compare r.alb dot in Glo454, Alb454, and in340 panels of Fig. 4). No signal above background was obtained with a ~800-nucleotide segment of the β-globin gene after infection with either virus (data not shown) (see Table 1). After 24 h of infection in the absence of E1A polypeptides (so that no virus DNA replication occurred), very active transcription was detected from several segments of DNA following the Alb454 promoter (see dots labeled IX, E1B, and r.alb, all of which represent part of the transcription unit governed by the albumin or globin promoters). By this time after infection, the rate of transcription of the albumin-containing recombinant transcription unit was even greater than that of the endogenous human

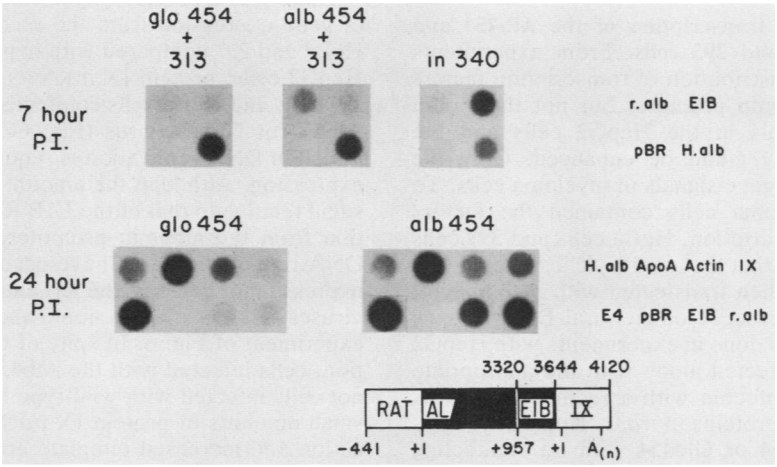

FIG. 4. Hybridization of ^{32}P-labeled nascent RNA prepared in nuclei isolated from virus-infected HepG2 cells. Samples in top row were from cells coinfected with *dl*313 and Glo454 or Alb454 or with in340 alone for 7 h. The samples in the bottom row were from cells infected for 24 h with Glo454 or Alb454 virus. The input of labeled RNA was 10^7 cpm; the DNA dots (5 μg each) of the indicated plasmid were rat albumin (r.alb −441 to +957; see Fig. 1), human apolipoprotein A (ApoA, a 2-kilobase cDNA), human albumin (H.alb, a 0.4-kilobase cDNA), chicken actin (a 1.5-kilobase cDNA), viral E4 (a 4-kilobase DNA from 89.7 to 100 map units), E1B (adenovirus nucleotides 3320 to 3644), protein IX (nucleotides 3644 to 4120), and pBR322 DNA (pBR). The pIX RNA initiation site is at nucleotide 3609 (5). P.I., Postinfection. The diagram shows the rat albumin (AL)-E1B transcription unit (solid box) and pIX transcription unit (open box).

TABLE 1. Relative transcription rates from albumin and globin promoters in recombinant adenoviruses after infection of Hela, 293, and HepG2 cells[a]

Expt	Plasmid DNA	Relative transcription (%)						
		HeLa		293		HepG2		
		Alb454	Glo454	Alb454	Glo454	Alb454	Glo454	in340
A	Rat albumin	2.4	—[b]	1.6	—	100	15	15
	E1B	16	2.6	147	153	100	3.6	1500
	Globin	—	1.2	1.2	1.2	—	—	—
B	Rat albumin	11	—			100	2.5	
	E1B	30	4.5			100	1.5	
	Protein IX	142	69			100	<1.0	
	Globin	—	3.1			—	<1.0	

[a] For comparison of transcription rates, the data in Fig. 4 and additional autoradiograms of various exposures were analyzed by densitometric scanning. Since the Alb454 virus in HepG2 cells gave the best transcriptional performance, the values in that cell type for the various DNA segments were scored as 100%. Experiment A is for cells infected for 7 h with E1A protein provided by coinfection, and experiment B is for cells infected for 24 h without E1A proteins. For each comparison within a given cell type, the value for each dot traced was normalized by using the actin signal obtained in each cell sample. The value for the actin dot in cells infected with various viruses did not vary more than 30 to 40% in various samples. For comparisons between different cell types at a particular time, all the transcriptional signals from a particular cell type were normalized to the signal obtained for the E4 dot. Again the three different cell types gave very similar E4 signals after 7 h of infection and also after 24 h of infection without E1A protein. The zero setting for the densitometer was the pBR322 signal. The values for globin transcription in HeLa and 293 cells are relative to those for rat albumin observed in HepG2 cells infected with Alb454 virus.

[b] —, No significant signal over background.

albumin gene (compare H.alb and r.alb dots, Fig. 4, bottom). This was true even though the virus DNA had not replicated. Thus, it appears that many more polymerases were active on the albumin sequences in the virus genome at 24 than at 7 h, even without E1A proteins.

Although synthesis from the β-globin promoter was not detected (Fig. 4, bottom row), the globin-containing virus successfully infected the hepatoma cells, as indicated by the strong transcriptional signal with the adenovirus E4 DNA.

E1A proteins stimulate transcription of the Alb454 and Glo454 viruses in HeLa and 293 cells. From experiments considered thus far, the distribution of transcription factors seemed limited: the albumin promoter but not the globin promoter gave low signals in the HepG2 cells and the albumin promoter activity could be enhanced. Only the immunoglobuin promoter gave signals in myeloma cells. To test whether only hepatoma cells contained the factors required for albumin transcription, HeLa cells and 293 cells were infected. It is known that these cells yield some mRNA from a variety of genes when transfected with recombinant DNA, especially in the presence of the viral E1A proteins (11, 16, 23, 33, 34). As was done in experiments with HepG2 cells, HeLa cells were infected alone with the appropriate recombinant virus or coinfected with a recombinant virus plus *dl*313 to supply E1A proteins in *trans*. Human 293 cells were infected with Alb454 or Glo454 with no coinfecting virus. Seven hours after infection, cytoplasmic RNA was isolated and assayed to detect RNA originating from the albumin or globin promoters. HeLa cells infected with Glo194 did not express RNA from the globin promoter, but did express RNA from the albumin promoter of Alb194 at perhaps 1/10 to 1/20 that seen in HepG2 cells (data not shown). Since E1B mRNAs are constitutively expressed in 293 cells, for detection of RNA directed by the albumin or globin promoters we used 5′ sp6 RNA probes specific for these cellular genes. The Alb194, Glo194, and Ig194 viruses did not express any RNAs in 293 cells that could be detected by the 5′ sp6 RNA assay (data not shown). Transcriptional analyses were also done with both cell types infected with the Alb194 and glo194 viruses, with no detection of a signal above background.

When 293 cells were infected for 7 or 10 h with either the Alb454 or Glo454 virus, albumin- and globin-specific RNAs could be scored from the appropriate start site by using 5′-end probes for both cell promoters (Fig. 5). The experiment in Fig. 5 was done in the presence of cytosine arabinoside (ara-C) to prevent any DNA replication; an earlier experiment at 7 h without ara-C gave the same results. Comparing the results of several experiments with 293 cells and HepG2 cells, we estimate that the Alb454 and Glo454 viruses caused the production of about 10 to 20% as much RNA in 293 cells as the Alb454 virus coinfected with *dl*313 in HepG2 cells. Compared to ablumin RNA in hepatoma cells or globin RNA in induced MEL erythroleukemia cells, the infected 293 cells produced only about 5% as much specific mRNA.

E1B-specific RNAs were generated by both the Alb454 and Glo454 viruses in HeLa cells, and this accumulation was further stimulated by E1A proteins provided by coinfection with *dl*313 virus (Fig. 6A). However, unlike the approximately equal pattern of albumin and globin promoter expression observed in 293 cells, globin promoter expression was reduced in HeLa cells by at least 10-fold (Fig. 6A, lane 3; unpublished data). This was not due to an inability of the Glo454 virus to infect HeLa cells, since viral E4 expression by both the Alb454 and Glo454 viruses in these cells was similar (data not shown). For direct comparison with HeLa cells, the expression of the albumin promoter of Alb454 virus in HepG2 cells alone or with *dl*313 virus is shown in Fig. 5B. From these comparisons, albumin expression in HepG2 cells, both stimulated by E1A protein and not stimulated, was seen to be at least 10-fold greater compared with that in HeLa cells and 293 cells under similar conditions (Fig. 6A and B).

There was a possibly significant distinction in the pattern of gene expression from the recombinant viruses between HeLa and 293 compared with hepatoma cells. In contrast to HepG2 cells, protein IX mRNAs could be detected in both 293 cells and HeLa cells coinfected with *dl*313 and either the Alb454 or Glo454 virus (Fig. 6C). During wild-type virus infection DNA replication is required for protein IX mRNA expression. Although the amount of protein IX mRNA was small relative to that of the E1B RNA arising from transcription from the albumin promoter, we suspected that early DNA replication might have occurred, leading to increased expression of genes in the left end of the Alb454 and Glo454 viruses. That is why, as noted above, ara-C was used in the experiment of Fig. 6. In spite of this block to DNA replication, cells infected with the Alb454 and Glo454 viruses, but not cells infected with wild-type in340 virus, still expressed small amounts of protein IX mRNA (Fig. 6C). Thus, replication and increased template copies in HeLa cells do not appear to be the explanation for the limited protein IX mRNA formation from the Alb454 and Glo454 viruses or for the expression from the albumin and globin promoters shown in Fig. 6. Rather, it appears that inclusions of the enhancer plus the E1A proteins may activate at least to some extent any promoter in the left end of the virus in these undifferentiated human cells.

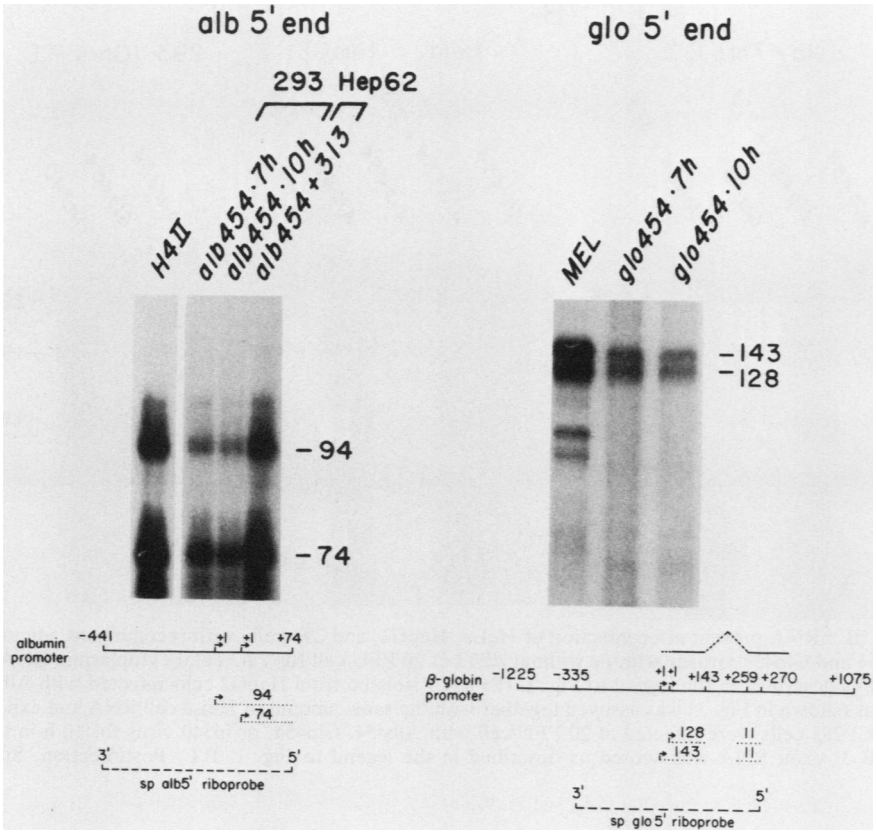

FIG. 5. sp6 assay for quantitation of albumin (alb)- and globin (glo)-specific RNA in 293 cells infected with Alb454 and Glo454 viruses. 293 cells were infected with 20 PFU of the appropriate virus for 7 or 10 h in the presence of ara-C (25 μg/ml), and total cytoplasmic RNA (15 μg) was isolated and hybridized to a ^{32}P-labeled sp6 RNA spanning the rat albumin promoter (−441 to +74) or the mouse β-globin promoter (−1225 to +1075). For comparison, dimethyl sulfoxide-induced mouse erythroleukemia cells (MEL), H4II (rat hepatoma), and Alb454- and *dl*313-infected HepG2 cells RNAs (15 μg) were also assayed.

Direct transcription measurements in undifferentiated cells. Finally, the rate of transcription from the albumin and globin promoters was measured in the isolated nuclei of infected HeLa cells and 293 cells (autoradiograms not shown; data shown in Table 1). Approximately the same amount of total nascent labeled RNA (~10^7 cpm) was used in the transcription analyses for hepatoma and HeLa or 293 cells. The autoradiographic exposures also were similar. The transcriptional signals for a commonly expressed cell gene (actin) could be directly compared within a cell line and for a virus gene (E4) between cell lines. These signals (which did not vary greatly between paired samples) were used to normalize the results of the transcriptional analysis (6). Therefore it was possible to compare transcription signals driven by either the albumin or globin expressions on the viral or cellular genome among the three cell types. The albumin promoter in HeLa cells infected with Alb454 virus for 24 h with no E1A protein was only about 11% as active as in hepatoma cells (Table 1). When E1A protein was present (coinfection of HeLa cells with Alb454 and *dl*313 virus, or Alb454 infection of 293 cells), the assay was made after 7 h of infection to avoid any possible complication from viral DNA replication. Again the transcription of albumin in hepatoma cells was 30 to 40 times higher than in HeLa or 293 cells (Table 1, line 1; see also r.alb dot in Fig. 4). However, the transcriptional signal from the albumin promoter in HeLa and 293 cells was significantly above the background (measured with plasmid DNA containing no insert). In addition, both the downstream E1B and protein IX signals were higher in HeLa and 293 cells due to expression of the protein IX promoter (demonstrated earlier by the presence of IX mRNA; Fig. 6). With the globin promoter there was a barely detectable transcriptional signal in HeLa cells coinfected with *dl*313 and Glo454 virus or 293 cells infected with Glo454 virus within the 7 h of infection (Table 1, line 3). By 24 h there was transcription from the globin promoter that was approximately one-third that of albumin promoter-driven transcription in HeLa cells (Table 1) and therefore about 1/30 that of the albumin promoter in hepatoma cells. Again, protein IX and E1B transcription were evident from the high signal in the downstream region only (Table 1, lines 2 and 3).

DISCUSSION

The experiments described in this and the accompanying paper (10) provide insight into three aspects of cell-specific transcriptional control: (i) the nature and distribution of cellular transcriptional factors, (ii) the interaction between enhancer and promoter elements, and (iii) the mechanism of action of the adenovirus E1A enhancer and E1A protein.

Differentiated cells respond specifically at transcriptional level. The cell-specific expression of the albumin promoter in HepG2 cells was not increased by E1A proteins alone but was increased in the presence of the E1A enhancer and

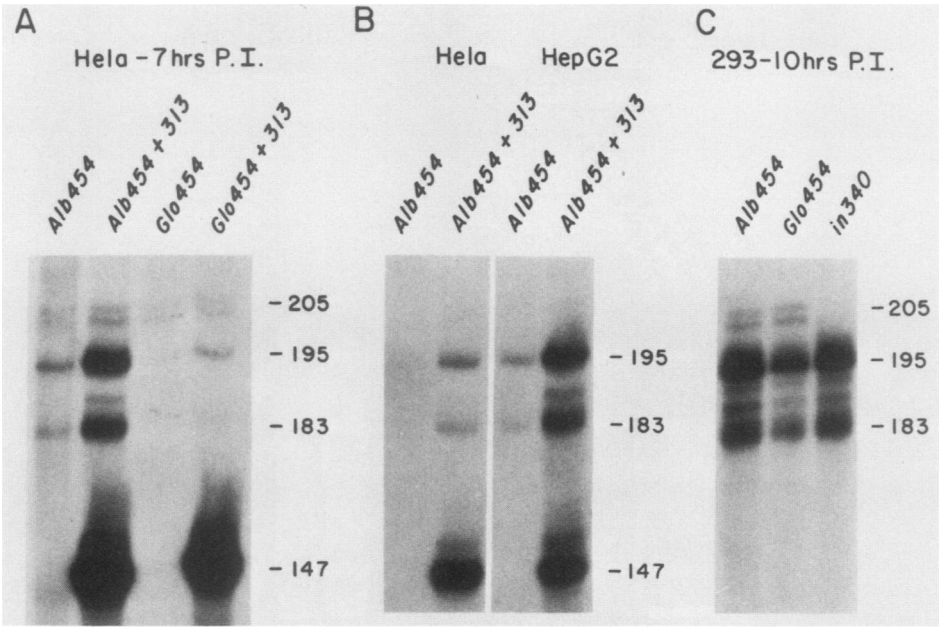

FIG. 6. Analysis of E1B mRNA present after infection of HeLa, HepG2, and 293 cells with recombinant adenoviruses. (A) HeLa cells were infected with Alb454 and Glo454 viruses with or without *dl*313 at 20 PFU/cell for 7 h. Total cytoplasmic RNA was isolated, and E1B 3′ exon RNA was scored as described in the legend to Fig. 1. (B) RNA isolated from HepG2 cells infected with Alb454 and Glo454 viruses with or without *dl*313 virus (shown in Fig. 1) was assayed together with the same amount of HeLa cell RNA and exposed to autoradiography for an identical period. (C) 293 cells were infected at 20 PFU/cell with Alb454, Glo454, or in340 virus for 10 h in the continuous presence of ara-C (25 μg/ml). E1B 3′ exon RNA was scored as described in the legend to Fig. 1. P.I., Postinfection. Sizes (in nucleotides) are indicated.

greatly increased by the presence of both. This increase was shown directly to be due to increased transcription. Moreover, globin transcription from the virus genome in HepG2 cells was not activated by either viral element, and expression from the immunoglobulin virus construct, which carries its own enhancer, was not stimulated by E1A protein in MPC11 cells (or in any other cell line). Therefore, in differentiated cells the viral enhancer will only boost the transcription rate if a tissue-specific factor(s) has established a low level of transcription. In all cases we should note that each of the three cellular promoter regions studied, rat albumin, mouse immunoglobulin, and mouse globin, could direct transcription from the correct RNA initiation site when situated on the viral genome.

If cell-specific transcription factors are present in hepatomas, why would the E1A enhancer function be required for maximal transcription? Perhaps the virus enhancer and its stimulatory E1A proteins are performing a function normally carried out in the liver cell by other sequences and proteins. Recent analysis of the chromatin structure of the active rat albumin gene in liver tissue has revealed three regions of DNase I hypersensitivity, located at positions −50, −200, and −2800 from the cap site (L. E. Babiss, A. Bennett, and J. E. Darnell, Proc. Natl. Acad. Sci., in press). The recombinant albumin viruses studied here contain only 441 nucleotides of upstream sequence and thus may be missing an enhancer that normally functions in the albumin locus. Enhancer elements have recently been localized upstream of the α-fetoprotein gene (14). Furthermore, Gonzalez and Nebert (15) have shown that the Harvey murine sarcoma virus enhancer can substitute for an upstream element responsible for transcriptional activation of the mouse P-450 gene. We are in the process of constructing additional recombinant viruses that extend the albumin genomic sequences to −3500 from the cap site in an effort to determine the importance of these sequences in determining the rate of albumin gene transcription.

Distribution and nature of albumin gene transcription factors. If there were strictly cell-specific positive-acting factors, why is there some albumin and globin transcription from the correct start site in HeLa and 293 cells? For example, in bacterial cells only the ara-C protein will promote high levels of arabinose operon transcription (20), and in yeast cells the Gal4 protein is the only protein that acts positively to stimulate the scattered genes encoding galactose-metabolizing enzymes (13, 28).

The activity of the albumin promoter in HeLa and 293 cells could imply the presence of (i) the same transcription factors as are present in hepatoma cells, (ii) a different set of transcription factors that nevertheless recognize the upstream sequences of the albumin gene, and (iii) reflect the absence of site-specific repressors present in cells that do not express a given tissue-specific gene.

Solution to the nature of potential positive- and *trans*-acting factors clearly requires that such factors be purified from each cell type in which that gene is active. Several positive-acting factors with different abilities to stimulate in vitro transcription of specific viral and certain widely transcribed genes (thymidine kinase, for example [21]) are now known from animal cells, but in vitro transcription that reflects the differentiated state have yet to be clearly demonstrated. In fact one of the early results of in vitro transcription was the demonstration that HeLa cell extracts would transcribe a variety of normally cell-specific genes (29).

However, the endogenous albumin and β-globin genes in

HeLa and 293 cells are clearly inactive, yet transcription of these promoters on the virus in these cells occurs. The factors that are responsible for transcription from the ablumin promoter on the virus DNA in HeLa and 293 cells clearly are somehow prevented from acting on cell chromosomes. It is possible that the chief cause of the higher rate of albumin transcription in HepG2 cells is the shielding of the albumin promoter from the repressive effects of some generalized inactivation factor present in other cell types? Experiments fusing hepatoma cells with undifferentiated cells (25) support the presence of negative-acting proteins that "extinguish" hepatocyte-specific function in undifferentiated cells, such as mouse fibroblasts. That such factors may be at least partially gene specific is suggested by the necessity for only a single mouse chromosome for extinction of rat albumin expression. Again, a satisfying answer to these questions awaits the isolation of transcriptional factors that can be assayed in vitro.

A final point arising from these experiments concerns the mode of action of the viral E1A enhancer element and its interactions with E1A proteins. In the experiments described in this paper, comparisons have been made with the enhancer on the viral genome upstream of the cell promoter. The E1A proteins were introduced by a coinfecting virus or were present as a constitutive product in 293 cells. First, we note that the viral enhancer alone is capable of increasing albumin RNA production in HepG2 cells. However, the E1A enhancer alone is not sufficient to activate any gene, since globin was not so activated. As mentioned, these results suggest that the E1A enhancer may work by increasing the access of already present transcriptional factors to the incoming gene. We do not know in this case (indeed it is unclear in the case of any enhancer) whether such a stimulation results from recruiting more templates into active and stable transcription complexes or from increasing the frequency of initiation on the same number of templates.

Another parameter of experiments designed to test E1A action on cell genes is the vector used. For example, Green et al. (16) showed that a human α-globin construct introduced into either HeLa or 293 cells was expressed at a similar level, suggesting no E1A protein stimulation. This differs from the results of Hearing and Shenk (19) with an α-globin gene on the viral chromosome; they found that E1A protein and enhancer were required for maximal β globin expression from the viral chromosome. In other experiments, the human β-globin promoter and a mouse preproinsulin promoter in plasmids were stimulated by E1A protein (11, 16) in the absence of the E1A enhancer sequences. In unpublished experiments we have found that all three promoters used in viruses in this study (albumin, β-globin, and immunoglobulin heavy chain) can be stimulated by E1A protein when they are part of a plasmid lacking the E1A enhancer. As noted above, however, in the virus genome albumin and globin require the E1A enhancer to function in HeLa and 293 cells. Thus, both the gene under study and the vector used to introduce it can have an important effect on responsiveness to the E1A protein and the requirement for the E1A enhancer to observe the E1A effects.

Several general points come from comparisons of the reported results and our results. (i) The E1A enhancer may only increase transcription of a gene if a compatible set of transcription factors for that gene is available to a cell. (ii) The activities of transcription factors (either positive or negative) are very likely different when promoters are on circular plasmids than with a linear adenovirus template. Which presents a more valid picture of how sequences in their native chromosomal locus are regulated is not clear. (iii) The cell sequences included within an adenovirus construct, particularly enhancer sequences, may help determine whether other proteins will have a stimulatory effect on transcription. (iv) The E1A proteins can have a direct stimulatory effect on transcription mediated by the E1A enhancer itself in a variety of cell types.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants CA-160006-12 and CA-18213-10 from the National Institutes of Health and by American Cancer Society grant MV2710. J.M.F. received a National Research Service Award (1-F32 AMO7338-02). L.E.B. is a Damon Runyon Fellow (DRG-794).

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