Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 9076–9080, December 1988
Cell Biology

Conditional enhancement of liver-specific gene transcription

(albumin gene transcription/hepatocyte cell culture/enhancer element/immortalization with simian virus 40)

KENNETH S. ZARET*, C. MICHAEL DIPERSIO, DAVID A. JACKSON, WILLIAM J. MONTIGNY, AND DEBRA L. WEINSTAT†

Section of Biochemistry, Box G, Brown University, Providence, RI 02912

Communicated by Philip W. Majerus, September 6, 1988 (received for review July 14, 1988)

ABSTRACT **We sought to develop a cell line in which liver-specific transcription could be induced at will, to facilitate the study of factors that cause hepatocyte-specific transcription of the serum albumin gene in mice. We therefore created the H2.35 cell line from mouse hepatocytes infected with a temperature-sensitive strain of simian virus 40. During routine propagation at the permissive temperature, H2.35 cells exhibit extremely low levels of albumin transcription and mRNA. Albumin mRNA increases at least 100-fold when H2.35 cells are cultured at the restrictive temperature and in serum-free medium on a collagen substratum; the two latter conditions maintain the differentiated state of primary hepatocyte cultures. Although a major cause of the mRNA increase is posttranscriptional, the transcription rates of albumin and other liver-specific genes increase significantly. Transient-transfection experiments demonstrated that an induction of transcription is caused by activation of an albumin upstream sequence that was previously shown to enhance liver-specific transcription in transgenic mice. Thus, hepatocyte differentiation appears to be maintained in part by extracellular signals that stimulate the activity of a tissue-specific enhancer element.**

Our interest is in factors that cause liver-specific transcription of the serum albumin gene in mice. The albumin gene is transcriptionally activated during the development of the fetal liver (1) and is transcribed constitutively and at a high level only in hepatocytes (2, 3). When hepatocytes are removed from the animal and cultured *in vitro*, the transcription rates of albumin and other liver-specific genes decrease dramatically (4, 5). This rapid dedifferentiation can be partially prevented by culturing the cells in a serum-free, hormonally defined medium (6, 7). Culture on a collagen matrix, rather than on plastic, also causes albumin mRNA levels to remain high (6), possibly due to a posttranscriptional mechanism (7). Thus, external cues appear to be required for maintenance of the differentiated state of hepatocytes; a subset of such cues could operate by stimulating regulatory elements that confer liver-specific transcription.

Pinkert *et al.* (8) have identified albumin regulatory sequences that function only in liver. When introduced into transgenic mice, a construct containing the mouse albumin promoter was essentially unexpressed (8). However, the addition of a DNA segment from 10 kilobases (kb) upstream of the albumin gene caused the promoter to be active at a high level only in the liver (8). The upstream element is DNase I-hypersensitive solely in liver chromatin (3, 8), suggesting that trans-acting factors causing its tissue-specific activity are present in adult hepatocytes. To investigate whether extracellular stimuli control the enhancer's activity, we needed to study albumin gene transcription in hepatocytes that were removed from the context of the animal.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Techniques are well established for isolating hepatocytes from mice and culturing them *in vitro* (reviewed in ref. 9); however, it is difficult to obtain large populations of homogeneous cells. Clonal homogeneity can be obtained by using continuously transformed, hepatocyte-derived cell lines (10, 11), but in such cells the transformed state may partially abrogate cell-specific transcription (10). We wanted to create a conditionally transformed mouse cell line expressing liver-specific functions, so that the relevant regulatory elements could be resolved in an isogenic background. Chou (12) demonstrated induction of albumin expression in a rat cell line derived from hepatocytes infected with a strain of simian virus 40 (SV40) that is temperature-sensitive for transformation. Although transcription rates were not determined, the cells expressed high steady-state levels of albumin mRNA at the restrictive temperature and in the presence of glucocorticoid hormone. Presumably, at the restrictive temperature the cells gained the potential to resemble primary hepatocytes. We therefore created a temperature-sensitive mouse hepatocyte-derived cell line to facilitate the study of mechanisms by which exogenous stimuli influence liver-specific transcription.

MATERIALS AND METHODS

Viral Infection and Creation of Cloned Cell Lines. Livers of 6-week-old female BALB/c mice were perfused with collagenase (4). Cells were collected and cultured at a density of 7×10^5 per 100-mm plate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT), 1 μM hydrocortisone, 10 μg of insulin per ml, and antibiotics (penicillin and streptomycin, each at 100 units/ml) (12). At least 50% of the adhered cells were binucleate hepatocytes. After 6 hr of incubation at 33°C, cells were infected with SV40 tsA255 (12) or mock-infected. Cells were cultured at 33°C in basal medium (12) (BM; DMEM supplemented with 4% FBS, 0.2 μM dexamethasone, and antibiotics), which was replaced thrice weekly. Two to 3 weeks postinfection, colonies that could not be subcloned arose over the monolayer of dying cells. By 4–5 weeks, colonies amenable to subcloning appeared. These were picked with cloning cylinders, dispersed with trypsin, and plated in BM at 33°C. Secondary colonies were picked, expanded to establish clonal cell lines, and stored frozen under liquid nitrogen. Mock-infected cells gave rise to a few sparse colonies that could not be subcloned.

Culture Conditions for DNA Transfection and RNA Preparation. For stable RNA analysis or transfections, H2.35 cells (10^6 per plate) were seeded onto 100-mm tissue culture plates. Plates either (*a*) contained BM and were incubated at 33°C or

Abbreviations: SV40, simian virus 40; T antigen, tumor antigen; RSV, Rous sarcoma virus; BM, basal medium; BM/s, supplemented BM; SFM, serum-free medium; FBS, fetal bovine serum.

*To whom reprint requests should be addressed.
†Present address: Department of Human Genetics, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510.

(*b*) contained 4 ml of a 1.35-mg/ml gel of rat tail type I collagen (Collaborative Research, Waltham, MA), prepared at 4°C in phosphate-buffered saline (PBS) containing 10 mM NaOH and gelled at 37°C for 45 min. The gels were incubated overnight at 39°C under 10 ml of BM/s [ref. 32; BM supplemented with insulin (10 μg/ml), triiodothyronine (50 ng/ml), and FBS (10% final concentration)], which was replaced with 10 ml of fresh BM/s immediately before the cells were added; this treatment dramatically enhanced plating efficiency. After the cells were cultured at 39°C for 5 hr, the medium was replaced with serum-free medium (SFM) prepared as described (6) but with dexamethasone at 0.2 μM.

For the RNA analysis in Fig. 2, all cells received two changes of medium over 4 days. For the 8-day time point, cells of 4-day cultures were released from the matrix with a 0.2-μm-filtered, 10-mg/ml solution of collagenase in DMEM, replated onto collagen in BM/s as described above, and then switched to SFM for an additional 4 days. For the transfections in Fig. 4, cells were seeded as described above and maintained in SFM or BM overnight, before addition of DNAs that had been precipitated with calcium phosphate (13). After 24 hr in the presence of DNA, cells were washed twice *in situ* with DMEM, fed with the designated media, and incubated for an additional 24 hr. The monolayers were rinsed twice with PBS, and a guanidinium isothiocyanate solution (14) was added to lyse the cells and solubilize the collagen gel. RNA was isolated from cell lysates by centrifugation through CsCl (14).

Analysis of RNA. Specific RNAs were quantitated either by electrophoresis in formaldehyde/agarose gels, followed by transfer to nitrocellulose and hybridization with nick-translated probes (15), or by hybridization with end-labeled synthetic oligonucleotides, primer extension with reverse transcriptase, and electrophoresis of the extension products in denaturing polyacrylamide gels, as described (3). Albumin mRNA levels were quantified by scanning different autoradiographic exposures of filters and gels with an LKB laser densitometer.

Nuclear Run-On Transcription Analysis. H2.35 cells were seeded at a density of 6×10^5 per 150-mm plastic plate in BM at 33°C, or at 3×10^6 per 150-mm plate containing collagen, as described above, and received a change of medium every 2 days. Cells were released from plastic dishes with a trypsin/EDTA solution, or from collagen gels with collagenase. Cells were washed twice in PBS, and nuclei were prepared and used for run-on transcription analysis as described (5); cytoplasmic RNA was isolated from the supernatants and assayed by primer extension. The [^{32}P]RNAs from the run-on assays were partially degraded with 0.1 M NaOH on ice, neutralized, and hybridized to 2-μg DNA samples of the designated probes immobilized on nitrocellulose. One-twenty-fifth amounts of the RNAs were used for separate hybridizations to DNA encoding ribosomal RNA. Specific hybrids were detected by autoradiography and quantitated by densitometry.

Recombinant Albumin Constructs. The mouse albumin promoter segment was constructed from an *Nco* I–*Hin*dIII fragment of the alb8 phage clone (16). The fragment was deleted at the 3′ end by digestion with exonuclease III and nuclease S1, to generate a segment spanning base pairs −787 to +8 with respect to the transcription start site. The albumin promoter fragment was fused to the thymidine kinase coding sequence (*tk*) (17) or the bacterial neomycin-resistance gene (*neo*) (18), to create the plasmids pAT2 and pAN2, respectively. For transfections, different albumin segments were inserted upstream of the −787 site. Fifteen micrograms of pAT2 or pAN2, or equimolar amounts of their derivatives, was transfected (13) per 10^6 cells. To control for transfection efficiency, we included 0.3 or 1.0 μg of the plasmid pRT1, which contains the Rous sarcoma virus long terminal repeat (RSV LTR) (19) fused to *tk*. The same *tk*-specific primer was used to quantitate expression from transfections with both the pAT2 and pRT1 plasmids; both *neo* and *tk* primers were used in the same hybridization reaction for analyses of pAN2 and pRT1.

RESULTS

Repression of Serum Albumin mRNA Levels in SV40-Infected Cells. Hepatocyte cultures were either infected with SV40 tsA255 (12) or mock-infected. After 12 days of culture at the permissive temperature (33°C), total cellular RNA was isolated from plates of confluent cells. Mock-infected cells expressed much lower levels of albumin mRNA than liver tissue, demonstrating dedifferentiation of the cells in culture (Fig. 1). The infected cells expressed even lower levels of albumin mRNA, relative to actin mRNA, at the permissive temperature. As expected, only the infected cells expressed SV40 mRNAs (Fig. 1).

Conditional Expression of Albumin mRNA. Many of the cloned, SV40-infected cell lines doubled at least once a day when cultured at 33°C but died in 2–3 days at 37°C or 39°C. One cell line, designated H2.35, survived at least 3 days at any of these temperatures in BM and, at early passage number, exhibited a 5-fold increase in albumin mRNA at the restrictive temperature (39°C; data not shown).

By 25–30 cell passages, albumin mRNA was no longer detectable in H2.35 cells maintained in BM on plastic dishes at 33°C (Fig. 2, lane 2). However, if the cells were then cultured for 4 days in SFM (adapted from ref. 6) on type I collagen gels at 39°C, albumin mRNA levels increased dramatically (Fig. 2, lane 3). When normalized to the essentially invariant actin mRNA levels (Fig. 2), albumin mRNA increased by >100-fold. Culture under these conditions for 8 days did not further enhance albumin expression (Fig. 2, lane 4). By comparison with a dilution of liver RNA (Fig. 2, lane 1), albumin mRNA in H2.35 cells was induced to about 0.02 the level in liver in 4 days. SV40 large tumor (T)-antigen mRNA increased over the 8-day period, presumably because inactivation of large T antigen at the restrictive temperature caused derepression of the SV40 early promoter (22, 23).

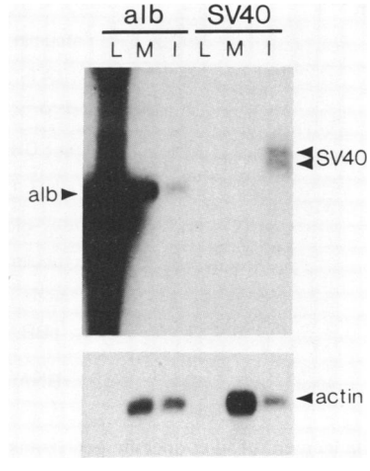

FIG. 1. Albumin mRNA levels are suppressed in SV40-infected cells. Three-microgram samples of total RNA from mouse liver (L) and from mock-infected (M) and SV40-infected (I) hepatocytes after 12 days in culture were electrophoresed in a denaturing agarose gel, blotted to nitrocellulose, and hybridized to nick-translated albumin cDNA (16) and SV40 DNA probes; the filters were exposed to x-ray film together (*Upper*). The position of the 2.2-kb albumin (alb) mRNA is shown at left. Probes were removed from the filters and the RNAs were hybridized to actin cDNA (20) as a control (*Lower*); actin mRNA levels are reproducibly low in intact liver.

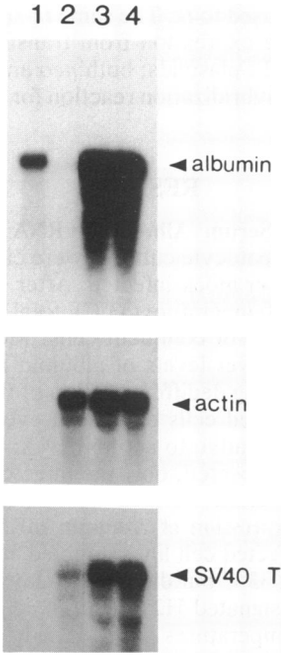

FIG. 2. Induction of albumin mRNA in H2.35 cells. RNA samples were analyzed as in the legend to Fig. 1. Lane 1: 10 ng of total liver RNA mixed with 4 μg of *Escherichia coli* tRNA. Lanes 2–4: 4-μg total RNA samples from H2.35 cells cultured in BM on plastic dishes at 33°C (lane 2) or cultured for 4 days (lane 3) or 8 days (lane 4) in SFM on a collagen gel matrix at 39°C. The same filter was probed sequentially with an albumin cDNA, an actin cDNA, and a DNA segment encoding SV40 large T antigen (21).

Induction of Liver-Specific Gene Transcription. To study the mechanism of albumin mRNA induction in H2.35 cells, we determined relative transcription rates of the albumin gene in cells cultured under different conditions for 4 days. The results of representative nuclear run-on transcription assays are seen in Fig. 3*a*. In H2.35 cells cultured in BM on plastic at 33°C, albumin transcription signals were consistently at the background level. By contrast, albumin was transcribed at a markedly higher level in H2.35 cells cultured in SFM on collagen at 39°C (Fig. 3*a*). The increase in transcription was selective, as actin and ribosomal RNA transcription rates were unaffected by culture conditions. Because the albumin transcription rate was below the level of detection under basal conditions, we could not quantify the extent of transcriptional induction. The induced transcription signal was sensitive to α-amanitin (1 μg/ml), demonstrating that it represented products of RNA polymerase II. Reproducible increases in transcription rate were also observed for liver-specific genes encoding phospho*enol*pyruvate carboxykinase (25), serine dehydratase (26), and a major urinary protein (livS-1) (27, 28), even though we did not attempt to optimize culture conditions for these hormonally responsive genes.

To ascertain that induced albumin transcription reflected use of the albumin promoter, transcript initiation sites were mapped by primer extension of the corresponding cytoplasmic RNAs. Albumin mRNA was undetectable in H2.35 cells cultured in BM at 33°C (Fig. 3*c*, lane 4). However, correctly initiated albumin transcripts were readily detectable in cells cultured in SFM on collagen at 39°C (lane 5). We conclude that accurate liver-specific transcription and posttranscriptional mRNA accumulation can be induced in H2.35 cells.

When H2.35 cells were cultured under inducing conditions for 4 days and then removed from the matrices with collagenase and cultured on plastic dishes in BM at 33°C for 9 days, albumin mRNA levels declined to basal levels (Fig. 3*c*, lane 6). When the "de-induced" cells were cultured again under inducing conditions for 4 days, high levels of albumin

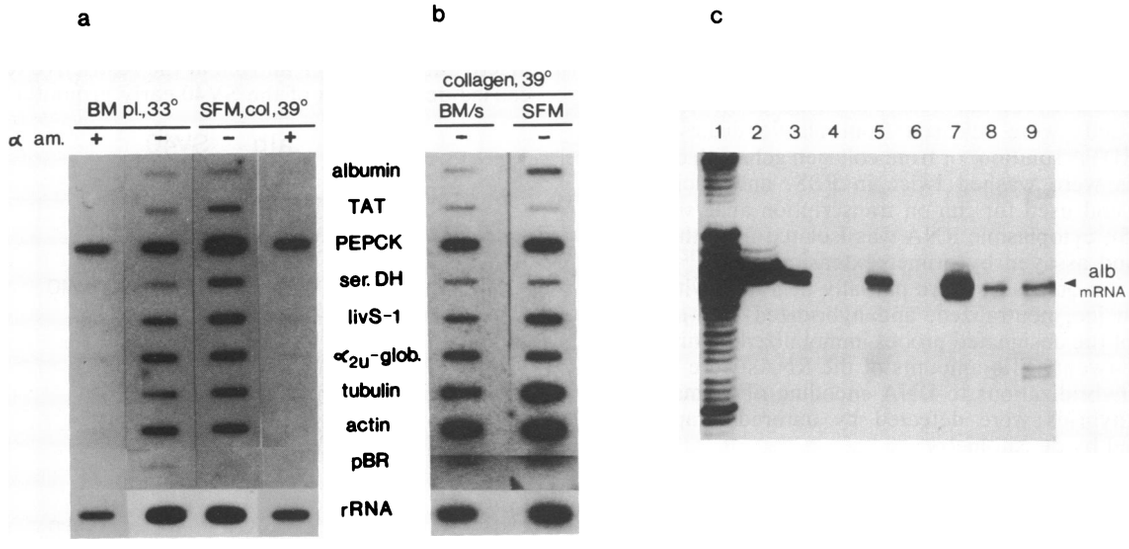

FIG. 3. Enhancement of liver-specific gene transcription in H2.35 cells. (*a*) Nuclear run-on transcription analyses of H2.35 cells cultured for 4 days in BM on plastic dishes (pl.) at 33°C, or in SFM on collagen gels (col.) at 39°C. Transcription reactions proceeded in the presence (+) or absence (−) of α-amanitin (1 μg/ml), and [^{32}P]RNAs (5 × 10^5 cpm, −; 2 × 10^5 cpm, +) were hybridized to the following cDNAs immobilized on nitrocellulose: albumin (16); TAT, tyrosine aminotransferase (24); PEPCK, phospho*enol*pyruvate carboxykinase (25); ser. DH, serine dehydratase (26); livS-1, a major urinary protein (27, 28); α_{2u}-glob., α_{2u}-globulin (29); β-tubulin (30); actin (20); pBR, control DNA for nonspecific hybridization; and DNA encoding 5S, 18S, and 28S ribosomal RNA (31). A portion of the PEPCK probe hybridized to a transcript that is probably due to RNA polymerase III; the transcription rate of this amanitin-insensitive transcript does not change under the different culture conditions. (*b*) Nuclear run-on transcription analyses of H2.35 cells cultured for 3 days on collagen gels at 39°C in either BM/s or SFM. A total of 4 × 10^6 cpm was used for each cDNA hybridization. (*c*) Primer extension analyses of albumin mRNAs. Lane 1: primer-generated DNA sequence ladder. Lanes 2 and 3: 250 and 50 ng, respectively, of total liver RNA mixed with 25 μg of *E. coli* tRNA. Lanes 4–9: 25 μg of cytoplasmic RNA from H2.35 cells cultured in BM (lane 4) or SFM (lane 5), from *a*; deinduced in BM on plastic at 33°C for 9 days (lane 6) and reinduced in SFM on collagen at 39°C for 4 days (lane 7); or cultured in BM/s (lane 8) or SFM (lane 9), from *b*.

mRNA were observed (lane 7); thus, the induction was reversible.

To test which culture components were critical for transcriptional induction, H2.35 cells were cultured for 3 days at the restrictive temperature on collagen gels either in SFM or in BM/s (BM/s permits much better growth on collagen than does unsupplemented BM). The albumin transcription rate was markedly greater than background only in H2.35 cells grown in SFM (Fig. 3*b*), even though steady-state albumin mRNA levels were similar under both conditions (Fig. 3*c*, lanes 8 and 9). Thus, culture at the restrictive temperature and on collagen was not sufficient to induce albumin transcription detectably, even though it caused an increase in accumulation of albumin mRNA. Our results agree with those of Reid, Darnell, and colleagues (5, 7) for primary hepatocytes and demonstrate that (*i*) serum either contains a component that represses transcription of albumin and other liver-specific genes or it lacks a positive-acting component and (*ii*) culture on type I collagen primarily stabilizes liver-specific mRNAs.

Activation of a Transcriptional Enhancer Element. Next, we studied the mechanism of transcriptional induction in H2.35 cells. The liver-specific enhancer described by Pinkert *et al.* (8) is an *Nhe* I–*Bam*HI DNA fragment, extending from −11.4 to −9.4 kb relative to the albumin transcription start site (Fig. 4*a*). We fused this fragment to an albumin promoter segment spanning the sequence from base pair −787 to base pair +8; *tk*, in pAT2, and *neo*, in pAN2, served as reporter genes. Constructs were transfected into H2.35 cells cultured either in BM on plastic at 33°C or in SFM on collagen at 39°C. As an internal control for transfection efficiency, we also included the plasmid pRT1. Two days later, transcript levels from cotransfected plasmids were quantitated by primer extension.

The data in Fig. 4*b* demonstrate that the albumin promoter initiated transcription from the authentic start site, when transfected into H2.35 cells under basal conditions. When transcript levels were normalized to pRT1, addition of the enhancer had no effect (lanes 3 and 4). By contrast, the enhancer stimulated transcription 2.5-fold in cells transfected in SFM on collagen at 39°C (lanes 5 and 6). To test whether the enhancer's effect was cell-type-specific in cultured cells, we transfected the same albumin sequences into mouse fibroblastic L cells cultured in SFM. The transfected albumin promoter was active in L cells; similarly, the β-globin (33) and growth hormone (34) promoters were also active upon transfection into L cells, despite inactivity of the endogenous genes. However, in L cells, the presence of the albumin enhancer had no effect on the albumin promoter (lanes 7 and 8). In transgenic mice, the distal *Nhe* I–*Sca* I subfragment (see Fig. 4*a*) of the enhancer stimulated the albumin promoter, but the proximal *Sca* I–*Bam*HI fragment did not (8). Neither fragment stimulated the promoter when transfected into H2.35 cells under basal conditions (lanes 9 and 10). However, in SFM on collagen at 39°C, the distal fragment stimulated transcription 4-fold, whereas the proximal fragment had no effect (lanes 11 and 12). Thus, the same enhancer fragments that were operative in transgenic mice are conditionally functional in H2.35 cells. We conclude that activation of a liver-specific enhancer element stimulates transcription in H2.35 cells.

DISCUSSION

We have been able to study the effects of exogenous factors on liver-specific transcription by using a mouse hepatocyte-derived cell line; H2.35 cells can be propagated in a dedifferentiated state and then partially induced to the differentiated state at will. This capability was achieved by employing techniques developed by Reid, Darnell, and colleagues (5–7), for maintaining liver-specific transcription in primary hepatocyte cultures. The biological significance of this induction is underscored by the enhanced transcription of a number of liver-specific genes in SFM, but not of genes encoding actin or ribosomal RNA. The induction of liver-specific transcription in H2.35 cells, and presumably its maintenance in hepatocyte cultures and in the animal, appears to require the presence of a particular complement of environmental cues.

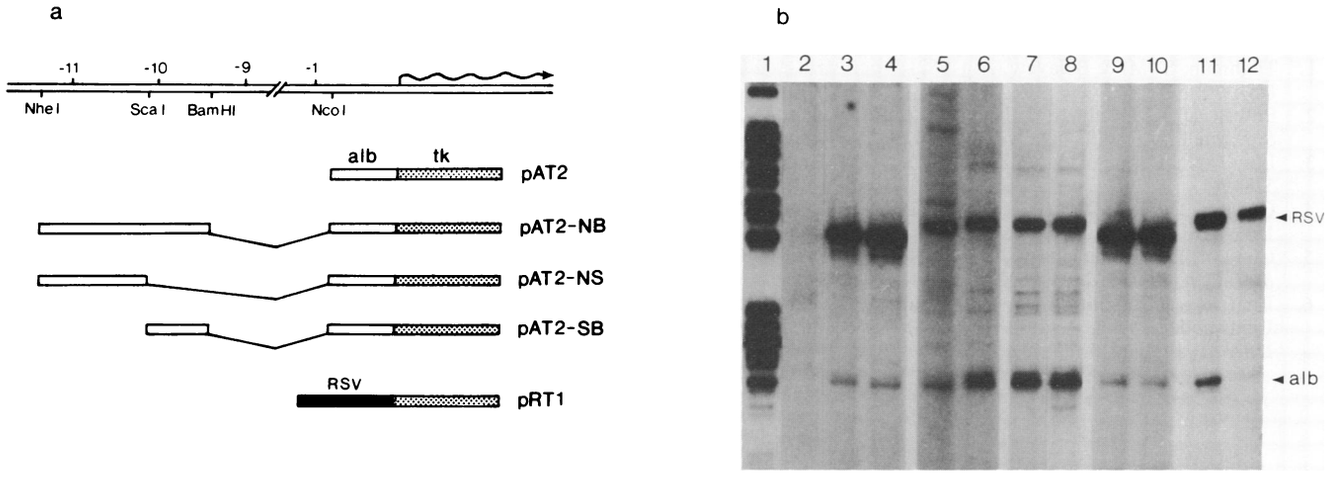

FIG. 4. Conditional activity of a liver-specific enhancer. (*a*) Mouse albumin DNA and recombinant contructs. Double line at the top indicates DNA of the albumin gene; relevant restriction sites are shown below. Wavy line indicates the transcribed region and numbers indicate distance (in kb) from the start site. Open boxes correspond to albumin DNA segments used in constructs for transfection. The albumin promoter segment was fused to either the *tk* coding sequence (stippled boxes) or the *neo* gene, to create the plasmid series pAT2 or pAN2 (latter not shown), respectively. All transfections included the control plasmid, pRT1. (*b*) Lane 1: primer-generated DNA sequence ladder from pAT2. Lane 2: primer extension of mock-transfected H2.35 cell RNA. Lanes 3–12: primer extensions of RNA from transfected cells; extension endpoints are indicated at the side of the autoradiograph. Lanes 3 and 4: H2.35 cells transfected with pAT2 and pAT2-NB, respectively, in BM on plastic at 33°C. Lanes 5 and 6: same DNAs as lanes 3 and 4, respectively, except that the H2.35 cells were transfected while in SFM on collagen gels at 39°C. Lanes 7 and 8: mouse L cells transfected with pAN2 and pAN2-NB while in SFM on plastic dishes. (The *neo* and *tk* primers yield identically sized extension products from pAN2 and pAT2, respectively. The pAN2 plasmid series is expressed identically to the pAT2 plasmids in H2.35 cells; data not shown). Lanes 9 and 10: H2.35 cells transfected in BM on plastic at 33°C with plasmids pAT2-NS and pAT2-SB, respectively. Lanes 11 and 12: same DNAs as lanes 9 and 10, except that the H2.35 cells were transfected in SFM on collagen at 39°C.

Here we have shown that part of the mechanism by which such environmental signals act is to stimulate the activity of a tissue-specific enhancer element. Thus, a regulatory element that confers cell specificity appears to gain part of its activity in response to cues in the cell environment. However, environmental stimuli are not sufficient to activate the enhancer, since it was inactive in L cells transfected in SFM. This could be due to an absence of the appropriate signal receptors in L cells; alternatively, trans-acting factors expressed in development may permit the enhancer to be active or to respond to external cues only in cells of the hepatocyte lineage. The appropriate environment, including hormones, growth factors, and extracellular matrix, could then stimulate enhancer activity by means of signal-transduction mechanisms within the cell. Indeed, hormonal influences on hepatocyte differentiation are well documented (9), and cell–cell interactions not only are required for hepatocyte formation in mouse development (35) but also can cause primary hepatocytes to retain more liver-specific characteristics in culture (36, 37).

The maximum induced level of albumin transcription in H2.35 cells, under the culture conditions tested to date, is about 1/20th the level in liver (with ribosomal RNA transcription as a comparison; see ref. 3). Similar culture conditions with primary hepatocytes result in an albumin transcription rate about 1/8th that in liver (7). It should be possible to use H2.35 cells to define conditions that further enhance hepatocyte-specific transcription, as well as to investigate trans-acting factors that must be inducible under the conditions described here. As a model system, H2.35 cells provide a genetically controlled context for studying factors that maintain the differentiated state of the hepatocyte.

We thank Janice Chou for providing tsA255 virus and advice; Philip Gruppuso for performing liver perfusions; Don Cleveland, Eva Derman, Daryl Granner, Keith Yamamoto, and especially Shirley Tilghman for cloned DNA probes; Kimberly Stevens for technical assistance; and Nelson Fausto, Paula Grabowski, Peter Shank, Patrice Milos, Jen-Kuei Liu, and Amy Plint for comments on the manuscript. The research was supported by grants to K.S.Z. from the National Institutes of Health (GM36477) and the Searle Scholars Program/Chicago Community Trust.

1. Tilghman, S. M. & Belayew, A. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5254–5257.
2. Powell, D. J., Friedman, J. M., Oulette, A. J., Krauter, K. S. & Darnell, J. E. (1984) *J. Mol. Biol.* **179**, 21–35.
3. Liu, J.-K., Bergman, Y. & Zaret, K. S. (1988) *Genes Dev.* **2**, 528–541.
4. Clayton, D. F. & Darnell, J. E., Jr. (1983) *Mol. Cell. Biol.* **3**, 1552–1561.
5. Clayton, D. F., Harrelson, A. L. & Darnell, J. E., Jr. (1985) *Mol. Cell. Biol.* **5**, 2623–2632.
6. Enat, R., Jefferson, D. M., Ruiz-Opazo, N., Gatmaitan, Z., Leinwand, L. A. & Reid, L. M. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1411–1415.
7. Jefferson, D. M., Clayton, D. F., Darnell, J. E., Jr., & Reid, L. M. (1984) *Mol. Cell. Biol.* **4**, 1929–1934.
8. Pinkert, C. A., Ornitz, D. M., Brinster, R. L. & Palmiter, R. D. (1987) *Genes Dev.* **1**, 268–276.
9. Reid, L. M. & Jefferson, D. M. (1984) *Hepatology* **4**, 548–559.
10. Clayton, D. F., Weiss, M. & Darnell, J. E., Jr. (1985) *Cell. Biol.* **5**, 2633–2641.
11. Woodworth, C., Secott, T. & Isom, H. C. (1986) *Cancer Res.* **46**, 4018–4026.
12. Chou, J. Y. (1983) *Mol. Cell. Biol.* **3**, 1013–1020.
13. Wigler, M., Sweet, R., Sim, G. K., Wold, B., Pellicer, A., Lacy, E., Maniatis, T., Silverstein, S. & Axel, R. (1979) *Cell* **16**, 777–785.
14. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
15. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201–5205.
16. Kioussis, D., Eiferman, F., van de Rijn, P., Gorin, M. B., Ingram, R. S. & Tilghman, S. M. (1981) *J. Biol. Chem.* **256**, 1960–1967.
17. McKnight, S. L. (1980) *Nucleic Acids Res.* **8**, 5949–5964.
18. Colbere-Garapin, F., Horodniceanu, F., Kourilsky, P. & Garapin, A.-C. (1981) *J. Mol. Biol.* **150**, 1–14.
19. Gorman, C. M., Merlino, G. T., Willingham, M. C., Pastan, I. & Howard, B. H. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6777–6781.
20. Cleveland, D. W., Lopata, M. A., MacDonald, R. J., Cowan, N. J., Rutter, W. J. & Kirschner, M. W. (1980) *Cell* **20**, 95–105.
21. Rio, D. C., Clark, S. G. & Tjian, R. (1985) *Science* **227**, 23–28.
22. DiMaio, D. & Nathans, D. (1982) *J. Mol. Biol.* **156**, 531–548.
23. Rio, D. C. & Tjian, R. (1983) *Cell* **32**, 1227–1240.
24. Scherer, G., Schmid, W., Strange, C. M., Röwekamp, W. & Schütz, G. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7205–7208.
25. Beale, E. G., Chrapkiewicz, N. B., Scoble, H. A., Metz, R. J., Quick, D. P., Noble, R. L., Donelson, J. E., Biemann, K. & Granner, D. K. (1985) *J. Biol. Chem.* **260**, 10748–10760.
26. Noda, C., Tomomura, M., Nakamura, T. & Ichihara, A. (1985) *Biochem. Biophys. Res. Commun.* **132**, 232–239.
27. Derman, E., Krauter, K., Walling, L., Weinberger, C., Ray, M. & Darnell, J. E., Jr. (1981) *Cell* **23**, 731–739.
28. Shahan, K., Gilmartin, M. & Derman, E. (1987) *Mol. Cell. Biol.* **7**, 1938–1946.
29. Unterman, R. D., Lynch, K. R., Nakhasi, H. L., Dolan, K. P., Hamilton, J. W., Cohn, D. V. & Feigelson, P. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3478–3482.
30. Valenzuela, P., Quiroga, M., Zaldivar, J., Rutter, W. J., Kirschner, M. W. & Cleveland, D. W. (1981) *Nature (London)* **289**, 650–655.
31. Renkawitz, R., Gerbi, S. A. & Glatzer, K. H. (1979) *Mol. Gen. Genet.* **173**, 1–13.
32. Miller, T. B., Jr., Garnache, A. & Cruz, J. (1984) *J. Biol. Chem.* **259**, 12470–12474.
33. Dierks, P., van Ooyen, A., Mantei, N. & Weissmann, C. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1411–1415.
34. Robins, D. M., Paek, I., Seeburg, P. H. & Axel, R. (1982) *Cell* **29**, 623–631.
35. Houssaint, E. (1980) *Cell Diff.* **9**, 269–279.
36. Fraslin, J.-M., Kneip, B., Vaulont, S., Glaise, D., Munnich, A. & Guguen-Guillouzo, C. (1985) *EMBO J.* **4**, 2487–2491.
37. Morin, O. & Normand, C. (1986) *J. Cell. Physiol.* **129**, 103–110.