5. T. T. Egelhoff, S. S. Brown, D. J. Manstein, J. A. Spudich, *Mol. Cell. Biol.* **9**, 1965 (1989).
6. J. Vieira and J. Messing, *Methods Enzymol.* **153**, 3 (1987).
7. A. De Lozanne and J. A. Spudich, *Science* **236**, 1086 (1987).
8. H. M. Warrick, A. De Lozanne, L. A. Leinwand, J. A. Spudich, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9433 (1986).
9. Cell line HS2210 was grown in 6-liter Erlenmeyer flasks, each containing 2.5 liters of HL5 medium. Cells were shaken on a gyratory shaker at 200 rpm at 22°C. MHF was typically purified starting with 40 liters of culture medium, which yielded about 250 g of cells. Cells were harvested at a density of 5×10^6 cells per milliliter by centrifugation for 7 min at 2500 rpm in an IEC PR-6000 centrifuge. To initiate development, cells were suspended at a density of 2×10^7 cells per milliliter in a solution containing 20 m*M* 2-(*N*-morpholino)ethanesulfonic acid (*p*H 6.8), 0.2 m*M* $CaCl_2$, and 2 m*M* $MgSO_4$ and shaken at 140 rpm for 4 hours. All remaining manipulations were performed at 4°C. The developed cells were washed once with a solution containing 10 m*M* tris (*p*H 8.0) and 1 m*M* EDTA and suspended in 1 ml of lysis buffer [50 m*M* Hepes (*p*H 7.5), 20 m*M* sodium pyrophosphate, 2 m*M* EDTA, 1 m*M* dithiothreitol (DTT), 30% (w/v) sucrose, 0.5 m*M* phenylmethylsulfonyl fluoride (PMSF), 5 m*M* benzamidine, *N*-tosyl-L-phenylalanine chloromethyl ketone (20 μg/ml), *Nα-p* tosyl-L-lysine chloromethyl ketone (20 μg/ml), and leupeptin (10 μg/ml)] per gram of cells. Cells were lysed by sonication with a Heat-System Ultrasonic model W 220-F sonicator equipped with a 0.75-inch flat tip. Unbroken cells and most of the cell debris were removed by centrifugation for 15 min at 40,000*g*. The supernatant was adjusted to 0.1*M* KCl using a 3*M* stock solution and then centrifuged at 100,000*g* for 3 hours. A key step in the purification scheme, resulting in a 140-fold enrichment in MHF, was the formation of an actomyosin pellet by removal of the sucrose from the resulting supernatant [M. Clarke and J. A. Spudich, *J. Mol. Biol.* **86**, 209 (1974)] and the specific release of MHF from this pellet by addition of the magnesium salt of ATP (Mg-ATP). The supernatant was dialyzed for 48 hours against 10 m*M* 1,4-piperazinediethanesulfonic acid (*p*H 6.9), 50 m*M* KCl, 2 m*M* EDTA, 0.5 m*M* DTT, 0.2% NaN_3, 1 m*M* benzamidine, and 0.5 m*M* PMSF. The actomyosin precipitate formed during dialysis was collected by centrifugation at 27,000*g* for 15 min and washed with HKE buffer [10 m*M* Hepes (*p*H 7.5), 50 m*M* KCl, 1 m*M* EDTA, 0.5 m*M* DTT, and 1 m*M* benzamidine]. The MHF wa solubilized by extracting the actomyosin pellet with HKE buffer containing 10 m*M* Mg-ATP in a Dounce-type homogenizer. The suspension was centrifuged at 48,000*g* for 15 min. To induce the formation of actin paracrystals, we increased the magnesium concentration of the supernatant to 12 m*M*. After incubation on ice for 30 min, 1 m*M* ATP was added to the now turbid solution, and the actin paracrystals were removed by centrifugation at 100,000*g* for 1 hour. A further fourfold enrichment in MHF was achieved by passing the resulting clear supernatant through a column (1.5 by 20 cm) containing a weak anion-exchanger matrix (Toyopearl DEAE-650 S) equilibrated with HKE buffer. The flow-through, containing the MHF, was collected and dialyzed overnight against HE buffer [20 m*M* Hepes (*p*H 7.5), 0.5 m*M* EDTA, 0.5 m*M* DTT, and 1 m*M* benzamidine]. The dialysate was then loaded onto a column (1.5 by 20 cm) containing a strong cation-exchanger matrix (Toyopearl SP-650 S) connected to a DEAE-650 S column (1.0 by 18 cm). Washing with HE buffer was continued as long as the flow-through showed absorbance at 280 nm. The SP-650 S column was then removed, and MHF was eluted from the DEAE-650 S column with a 75-ml gradient from 0 to 500 m*M* KCl in HE buffer. This combined cation-anion exchanger step led to a further fourfold enrichment for MHF. After this step the MHF could be used successfully both for the decoration of actin filaments and in an in vitro movement assay. Traces of impurities could be removed by passing the MHF over a Superose 12 gel filtration column.
10. G. Peltz, J. A. Spudich, P. Parham, *J. Cell Biol.* **100**, 1016 (1985).
11. D. J. Manstein, unpublished observations.
12. P. B. Moore *et al.*, *J. Mol. Biol.* **42**, 1 (1970).
13. Actin-activated ATPase activity was measured by adding 10 μg of MHF to 0.5 ml of assay solution containing 17 m*M* KCl, 2.7 m*M* $MgCl_2$, 0.7 m*M* EGTA, 0.7 m*M* DTT, 17 m*M* Hepes (*p*H 7.4), and actin. The final concentrations of actin in the assay solution were 0, 1, 2.5, 5, 10, and 20 μ*M*. All assays were performed at 30°C. Samples (100 μl) were removed after 5, 10, 15, and 20 min, added to 50 μl of stop-buffer containing 13.3% (w/v) SDS and 0.12*M* EDTA, and mixed vigorously. The release of phosphate was then determined by addition of 350 μl of freshly prepared developer solution [0.5*M* H_2SO_4, 0.5*M* ammonium molybdate, and 0.5% (w/v) $FeSO_4$]. The absorbance of 550 nm was measured [H. D. White, *Methods Enzymol.* **85**, 698 (1982)]. ATPase activity in the presence of Ca^{2+} was measured with 10 μg of MHF in a 0.5-ml assay volume. The assay solution contained 0.6*M* KCl, 5 m*M* $CaCl_2$, 4 m*M* ATP, and 10 m*M* Hepes (*p*H 7.4). ATPase activity in the presence of EDTA was measured in a similar way except that 25 μg of MHF was used and the assay solution contained 0.6*M* KCl, 5 m*M* EDTA, 4 m*M* ATP, and 10 m*M* Hepes (*p*H 7.4).
14. S. C. Mockrin and J. A. Spudich, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2321 (1976).
15. S. J. Kron and J. A. Spudich, *ibid.* **83**, 6272 (1986).
16. K. M. Ruppel, T. T. Egelhoff, J. A. Spudich, *J. Cell Biol.* **107**, 260 (abstr.) (1989).
17. H. E. Huxley, *J. Mol. Biol.* **7**, 281 (1963).
18. We thank E. Kubalek for providing micrographs showing MHF-decorated actin filaments; J. W. La-Claire II and T. Q. P. Uyeda for help with the in vitro motility assay; T. A. Mietzner for the gift of peptide antibody P5020; D. A. Kaiser and T. D. Pollard for the gift of antibody M II.42; and T. T. Egelhoff for his help in finding suitable expression vectors for *Dictyostelium*. Supported by NIH grant GM 33289 (J.A.S.). D.J.M was a recipient of a fellowship from the Deutsche Forschungsgemeinschaft (Ma 1081-2/2).

20 June 1989; accepted 12 September 1989

A Liver-Specific Enhancer in the Core Promoter Region of Human Hepatitis B Virus

JIING-KUAN YEE

An 88–base pair fragment in the core promoter of the human hepatitis B virus (HBV) contains a functional promoter and a strong liver-specific enhancer. This enhancer functions in human hepatoma cells, where it is much more active than the previously described HBV enhancer in stimulating expression of the linked bacterial chloramphenicol acetyltransferase gene expressed from heterologous promoters. Studies of the role of this enhancer-promoter in HBV may help to clarify mechanisms of gene expression in cells infected with HBV and the role of the virus in the pathogenesis of hepatitis and hepatocellular carcinoma.

HEPATITIS B VIRUS (HBV) IS A human hepatotropic virus that has infected approximately 200 million people worldwide. HBV causes either an acute or a chronic viral hepatitis, and chronic active hepatitis and liver cirrhosis are major causes of mortality. Furthermore, epidemiological studies have shown that infection with HBV is closely associated with an increased risk of primary hepatocellular carcinoma, one of the most common cancers in the world (*1*). Further evidence supporting the tumorigenic role of chronic HBV infection comes from the high incidence of hepatoma in woodchucks infected with woodchuck hepatitis virus (WHV) (*2*). Two years after initial viral infection, almost all of the animals developed hepatoma. However, since the precise role of HBV in hepatocellular tumorigenesis remains unclear, it is particularly important to elucidate the mechanisms that control the HBV life cycle and regulate its gene expression.

The principal site of clinical pathology for HBV is the liver, and HBV actively replicates in human hepatocytes (*3*). Two major HBV-specific mRNA species have been detected in the liver of HBV-infected chimpanzees (*4*); a subgenomic 2.1-kb RNA encoding the major surface antigen (HBsAg) and a 3.5-kb terminally redundant RNA, slightly longer than genome length, encoding the core antigen and possibly the DNA polymerase. The latter transcript is ultimately packaged into the viral nucleocapsid and serves as a template for viral DNA synthesis. A sequence resembling the late promoter of SV40 may direct the synthesis of the 2.1-kb RNA (*4*), but the promoter for the 3.5-kb RNA has not been identified. The 3.5-kb genomic transcript has never been detected in nonhepatic cells transfected with HBV DNA, but it has been found in well-differentiated human hepatoma cell lines (Hep G2, HuH6, and HuH7) transfected by the cloned HBV genome (*5*), suggesting that liver-specific factors are needed for correct transcription from the core promoter. In contrast, the 2.1-kb subgenomic HBV mRNA has been observed in a wide variety of transfected cell lines of diverse tissue and species origin (*6*), suggesting a relative lack of tissue specificity for the promoter of the

Department of Pediatrics, M-034, School of Medicine, University of California, San Diego, La Jolla, CA 92093.

Table 1. Assays of transient expression of the core promoter deletion mutants in transfected cells. Plasmid DNAs were introduced into cells by the method of calcium phosphate–mediated coprecipitation (*10*). CAT assays were performed 48 hours after transfection as described previously (*11*). The relative CAT activity of each construct was calculated as a percentage of that of pTKCAT. The values are the means of two to five independent experiments ± standard deviation (SD).

Plasmid	Relative CAT activity (SD)			
	BHK	CV1	HuH6	Hep G2
pTKCAT	1	1	1	1
pCCAT1	0.016 (0.003)	0.004 (0.001)	16 (7)	3.8 (0.2)
pΔCCAT1	0.004 (0.001)	0 (0)	18 (7)	0.5 (0.2)
pΔCCAT2	0.013 (0)	0.004 (0.001)	14 (2)	1.1 (0.1)
pΔCCAT4	0.010 (0.004)	0.009 (0.003)	13 (3)	1.5 (0.7)
pΔCCAT7	0.093 (0.009)	0.055 (0.002)	43 (4)	3.5 (0.6)
pSKCAT	0.015 (0.008)	0.010 (0.001)	0 (0)	0 (0)

HBsAg gene.

A transcriptional enhancer has previously been identified within a 200-bp region approximately 600-bp upstream from the transcriptional initiation sites of the 3.5-kb message (Fig. 1) (*7*). Enhancement of transcription by this fragment was modest in nonhepatic cells, but was substantially greater in several cultured hepatoma cells. However, the same HBV enhancer activated transcription in a relatively cell-independent manner when it was located either 5′ to the promoter or in the 3′ untranslated region of gene constructs (*8*). These latter results argue against a role for this enhancer in directing tissue-specific expression of HBV genes and raise the possibility that other regulatory sequences may determine the liver-specific expression of the 3.5-kb genomic message. Because of the importance of the 3.5-kb transcripts in the life cycle and pathogenicity of the virus, we sought to identify regulatory sequences in the core promoter and to determine the relation between the core promoter and the HBV enhancer.

In order to identify the sequences that regulate the synthesis of the 3.5-kb transcripts, I isolated an 844-bp Rsa I fragment from the cloned HBV DNA of subtype adr (Fig. 1) (*9*). This fragment spans the region containing the previously described HBV enhancer as well as the region between the enhancer and the ATG initiation codon of core antigen. Fragments containing a series of deletions from the 5′ end of the 844-bp fragment were isolated by restriction enzyme digestion and linked to the chloramphenicol acetyltransferase (CAT) gene as illustrated in Fig. 1. The effect of these deletions was analyzed by transient expression assays of CAT activity after calcium phosphate–mediated transfection of each plasmid into Hep G2 and HuH6 cells (*10*). Several cell lines, including baby hamster kidney (BHK) cells, monkey CV1 cells, and HeLa cells, were used as nonliver controls. The CAT activity of each deletion mutant was measured (*11*) and normalized to CAT expression from control plasmid pTKCAT, in which the CAT gene is expressed from the promoter of the herpes simplex virus (HSV) thymidine kinase (TK) gene (*12*) (Table 1).

In both transfected BHK cells and CV1 cells, the full-length Rsa I fragment present in pCCAT1 has very little promoter activity relative to that of the pTKCAT control. Deletion of the HBV enhancer to produce pΔCCAT1 resulted in one-fourth as much CAT activity in BHK cells and undetectable levels of CAT activity in CV1 cells. Further deletion in mutants pΔCCAT2 and pΔCCAT4 resulted in a modest increase in CAT activity compared to that of the pΔCCAT1. The deletion mutant pΔCCAT7, which contains only 88 bp of the HBV core promoter, showed more than 20 times as much CAT activity when compared with that of the pΔCCAT1 in BHK cells, suggesting that a cis-acting repressing element may exist between the Bam HI site at position 1272 and the Hinc II site at position 1554 that blocks or diminishes transcription from the core promoter in BHK cells and CV1 cells. The plasmid pSKCAT, which contains the CAT gene and no HBV sequences, had low but detectable levels of CAT activity in both BHK cells and CV1 cells. Similar studies were performed in HeLa cells, and none of the constructs containing the core promoter fragments showed any detectable levels of CAT activity (*13*). In contrast, in HuH6 and Hep G2 cells, the plasmid pCCAT1 as well as all the plasmids missing the previously described enhancer showed high levels of CAT activity when compared with that of the pTKCAT control. However, in transiently transfected Hep G2 cells, the promoter activity of each HBV fragment was 1/4 to 1/35 that found in HuH6 cells, suggesting that the cellular factors involved in the regulation of liver-specific gene expression from the core promoter may be different in the two human hepatoma lines. No CAT activity could be detected in pSKCAT-transfected hepatoma cells.

The 88-bp Hinc II–Rsa I fragment of the core promoter in the construct pΔCCAT7 is more than 40 times more active than the control pTKCAT in transfected HuH6 cells (Table 1). In order to determine if this fragment has the properties of a liver-specific enhancer in hepatoma cells, it was introduced in both orientations into pTKCAT either immediately upstream from the TK promoter (plasmids pCTKCAT2 and pCTKCAT3) or downstream from the CAT gene (plasmids pTKCATC1 and pTKCATC2) (Fig. 2A). Both transiently transfected HuH6 cells and Hep G2 cells expressed high levels of CAT activity irrespective to the positions and the orientations of the HBV sequence when compared with that of the control pTKCAT (Table 2). This increase in CAT activity was liver-specific, since no enhancement was observed in BHK or CV1 cells with any of these plasmids. When transcription initiation sites for the constructs pTKCATC1 and pTKCATC2 were mapped by primer extension analysis, enhanced transcription from the correct initiation sites of the HSV TK promoter was clearly seen in transfected HuH6 cells, and no enhancement could be observed in transfected BHK cells (Fig. 3). In Hep G2 cells, the 88-bp fragment was 1/5 to 1/10 as effective in stimulating CAT expression

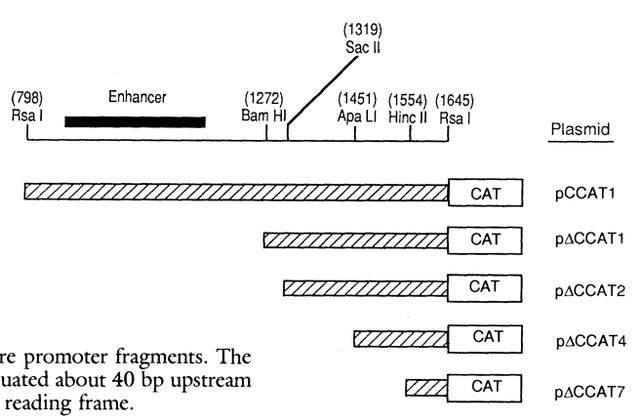

Fig. 1. Construction of the core deletion mutants. A detailed map of the core promoter region is shown. The sites of restriction enzymes used to construct the deletion mutants are shown together with their positions in the HBV genome (*9*). The closed bar represents the approximate position of the previously mapped HBV enhancer, and the hatched bars indicate the location and size of the HBV core promoter fragments. The Rsa I site at position 1645 is situated about 40 bp upstream from the start of the core open reading frame.

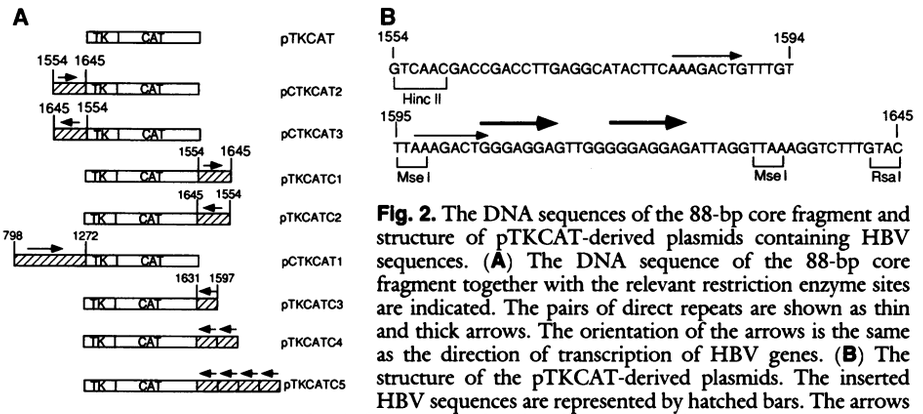

Fig. 2. The DNA sequences of the 88-bp core fragment and structure of pTKCAT-derived plasmids containing HBV sequences. (**A**) The DNA sequence of the 88-bp core fragment together with the relevant restriction enzyme sites are indicated. The pairs of direct repeats are shown as thin and thick arrows. The orientation of the arrows is the same as the direction of transcription of HBV genes. (**B**) The structure of the pTKCAT-derived plasmids. The inserted HBV sequences are represented by hatched bars. The arrows indicate the relative orientation of each fragment to the direction of transcription of HBV genes.

from the TK promoter as in HuH6 cells. In contrast, the construct pCTKCAT1, which contains the previously identified HBV enhancer inserted upstream from the TK promoter, showed little difference in CAT expression in transfected Hep G2 cells or HuH6 cells (Table 2). Similar to the results with the HSV TK promoter, the 88-bp fragment efficiently directed hepatoma-specific CAT expression from an enhancerless SV40 early promoter when it was inserted immediately downstream of the CAT gene (*13*). As a control, the 103-bp Apa L1–Hinc II fragment of the core promoter (which does not contain the 88-bp fragment) was inserted in both orientations into pTKCAT immediately upstream from the TK promoter; no significant increase in CAT activity relative to that of the pTKCAT could be observed either in HuH6 or BHK cells (*13*). Thus, the 88-bp core fragment is able to function as a tissue-specific enhancer and can activate transcription from heterologous promoters in an orientation- and position-independent manner.

The nucleotide sequence of the 88-bp core fragment is shown in Fig. 2B. A striking feature of this region is the presence of two pairs of direct 8-bp, partially overlapping repeats. To dissect the enhancer activity further, we inserted one or two copies of the 35-bp Mse I segment extending from 1597 to 1631 containing one copy of the first direct repeat and both copies of the second direct repeat into the construct pTKCAT to produce plasmids pTKCATC3 and pTKCATC4, respectively (Fig. 2A). In HuH6 cells, expression of the CAT gene from the TK promoter in pTKCATC3 was stimulated about threefold by inclusion of one copy of the Mse I segment, whereas insertion of two tandem copies of the Mse I segment in pTKCATC4 stimulated CAT expression greater than 25 times that of the TK promoter (Table 2). Four tandem copies of the Mse I segment in pTKCATC5 further increased CAT expression only slightly relative to that of pTKCATC4. The enhancement of CAT activity in pTKCATC4- or pTKCATC5-transfected HuH6 cells was at least 1/5 as much as that in pTKCATC2-transfected HuH6 cells (Table 2), indicating that other portions of the 88-bp core fragment are crucial for full enhancer activity. None of these plasmids containing the Mse I segment showed enhanced levels of CAT activity in nonhepatic cells compared with that of the pTKCAT control, indicating that the enhancer activity in this segment is liver-specific. Although the complete 88-bp fragment stimulates CAT expression in Hep G2 cells, none of the constructs containing the Mse I fragment showed any increased level of CAT activity in transfected Hep G2 cells.

The relatively low enhancer activity of the 88-bp fragment in the Hep G2 cell compared with that in the HuH6 cell may reflect differences in the availability of cellular transcription factors that interact with separate motifs within the 88-bp region in the two cell types. Previously, stable transformants that produce replicative intermediates and Dane particle–like structures have been isolated from Hep G2 cells and HuH6 cells after transfection with full-length HBV DNA (*5*). However, in stable HuH6 clones the relative abundances of the 3.5-kb pregenomic RNA and 2.1-kb RNA are about equivalent, whereas in Hep G2 cells the 3.5-kb message is less abundant than is the 2.1-kb message (*5*). This may be due to the

Table 2. Activation of transcription from the HSV TK promoter by the core enhancer relative to the level of gene expression from control plasmid pTKCAT. DNA transfection and CAT assays were performed as described in Table 1. The values are the means of two to five independent experiments ± standard deviation.

Plasmid	Relative CAT activity (SD)			
	BHK	CV1	HuH6	Hep G2
pTKCAT	1	1	1	1
pCTKCAT2	0.4 (0.1)	0.9 (0.2)	153 (20)	37 (5)
pCTKCAT3	1.0 (0.2)	0.8 (0.2)	144 (2)	30 (7)
pTKCATC1	0.9 (0.2)	1.1 (0.3)	274 (34)	26 (3)
pTKCATC2	0.6 (0.2)	1.0 (0.1)	228 (2)	53 (10)
pCTKCAT1	1.6 (0.4)	3.2 (0.8)	14.5 (2.1)	12.2 (2.6)
pTKCATC3	1.4 (0.3)	0.7 (0)	2.7 (0.3)	0.6 (0.1)
pTKCATC4	2.0 (0.2)	1.1 (0.6)	26.4 (2)	0.8 (0.2)
pTKCATC5	1.4 (0.2)	1.7 (0.3)	31.5 (2)	1.2 (0.4)

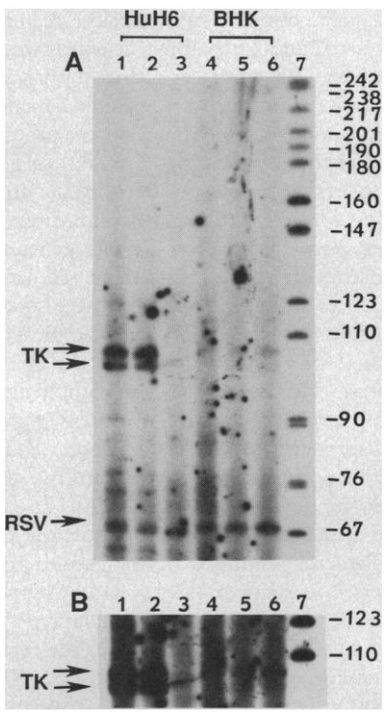

Fig. 3. Primer extension analysis of transcripts expressed in plasmids containing the core enhancer. The HuH6 cells and the BHK cells were transfected with 15 μg of pTKCATC1 (lanes 1 and 4), pTKCATC2 (lanes 2 and 5), or pTKCAT (lanes 3 and 6) together with 5 μg of pRSVCAT (*17*) as a control for the efficiency of transfection. A labeled 27-base CAT primer was used to hybridize with RNAs isolated from transfected cells, and the primer extension assay was performed as described in (*18*). Arrows labeled TK and RSV represent the RNAs correctly initiated at the TK and RSV promoters, respectively. End-labeled DNA fragments of pBR322 DNA digested with Msp I are shown in lane 7 as size markers. (**A**) The gel was exposed for 18 hours. (**B**) The same gel in (A), exposed for 72 hours, and only the area containing the correctly initiated RNA from the TK promoter is shown.

differential use of the core promoter by the two cell lines, an explanation that would be consistent with our observation that the enhancer in the 88-bp core fragment is more active in HuH6 cells than that in Hep G2 cells. Since Hep G2 and HuH6 cells may represent hepatocytes arrested at different stages of differentiation, as indicated by the fact that they express different sets of liver-specific proteins (*14*), this core enhancer may be developmentally regulated. Honigwachs *et al.* have recently shown that a similar core fragment failed to activate CAT expression in hepSK cells, a de-differentiated human liver line (*15*). Together, these results raise the interesting possibility that the absence of HBV replication and core gene expression observed in advanced hepatocellular carcinoma could be due to the decrease of the core enhancer activity in proportion to the de-differentiation of the infected hepatocytes.

It has been suggested that the previously described HBV enhancer may determine in part the liver-specific expression of the HBV pregenomic RNA (*16*). The results presented here indicate that the previously described enhancer does indeed function in human hepatoma cells, but it also stimulates CAT expression in nonhepatic cells. In contrast, activity of the enhancer located in the 88-bp core promoter region is restricted to human hepatoma cells. Since the 88-bp fragment by itself is sufficient to direct efficient CAT expression in hepatoma cells, it obviously also contains a functional promoter which, together with the tissue-specific enhancer, may account for the liver specificity of HBV gene expression. These studies of the HBV core promoter have demonstrated that a liver-specific enhancer activates expression from the core promoter in highly differentiated hepatoma cells. Deletion analysis of the core promoter region also suggests that a negative element in the core promoter may account for the extremely low activity of this promoter in nonhepatic cells. Such a combination of positive and negative regulatory effect may attribute to the highly liver-specific expression of the HBV 3.5-kb pregenomic RNA. The discovery of these regulatory signals in HBV should allow more informative studies on the regulation of HBV expression and replication in infected cells and the role of HBV in the development of hepatic malignancies.

REFERENCES AND NOTES

1. R. P. Beasley, C.-C. Lin, L.-Y. Hwang, C.-S. Chien, *Lancet* **ii**, 1129 (1981).
2. J. Summers, J. M. Smolec, R. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4533 (1978); P. Chen *et al.*, *ibid.* **83**, 8774 (1986).
3. T. Ochiya *et al.*, *ibid.* **86**, 1875 (1989).
4. R. Cattaneo, H. Will, N. Hernandez, H. Schaller, *Nature* **305**, 336 (1983).
5. C. Sureau, J.-L. Romet-Lemonne, J. I. Mullius, M. Essex, *Cell* **47**, 37 (1986); T. Tsurimoto, A. Fujiyama, K. Matsubara, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 444 (1987); K. Yaginuma, Y. Shirakata, M. Kobayashi, K. Koike, *ibid.*, p. 2678.
6. D. N. Standring, W. J. Rutter, H. E. Varmus, D. Ganem, *J. Virol.* **50**, 563 (1984).
7. Y. Shaul, W. J. Rutter, O. Laub, *EMBO J.* **4**, 427 (1985); A. Tognoni, R. Cattaneo, E. Serfling, W. Schaffner, *Nucleic Acids Res.* **13**, 7457 (1985).
8. J. L. Vannice and A. D. Levinson, *J. Virol.* **62**, 1305 (1988).
9. A. Fujiyama *et al.*, *Nucleic Acids Res.* **13**, 4601 (1983).
10. F. L. Graham and A. J. van der Eb, *Virology* **52**, 456 (1973).
11. M. J. Sleigh, *Anal. Biochem.* **156**, 251 (1986).
12. S. L. McKnight and R. Kingsbury, *Science* **217**, 316 (1982).
13. J. K. Yee, unpublished data.
14. B. B. Knowles, D. B. Searls, D. P. Aden, in *Advances in Hepatitis Research*, F. V. Chisari, Ed. (Masson, New York, 1984), pp. 196–202.
15. J. Honigwachs, O. Faktor, R. Dikstein, Y. Shaul, O. Laub, *J. Virol.* **63**, 919 (1989).
16. P. Tiollais, C. Pourcel, A. Dejean, *Nature* **317**, 489 (1985).
17. C. M. Gorman, G. T. Merlino, M. C. Willingham, I. Pastan, B. H. Howard, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6777 (1982).
18. S. L. McKnight, E. R. Gravis, R. Kingsbury, R. Axel, *Cell* **25**, 385 (1981).

19. I thank K. Matsubara for providing the cloned HBV DNA and the HuH6 line, and A. Miyanohara and T. Friedmann for many valuable discussions during this work.

3 May 1989; accepted 13 September 1989

Initiation by Yeast RNA Polymerase II at the Adenoviral Major Late Promoter in Vitro

NEAL F. LUE, PETER M. FLANAGAN, KATSUNORI SUGIMOTO, ROGER D. KORNBERG

Transcription of the yeast *CYC1* promoter fused to a sequence lacking guanosine residues provided a rapid, sensitive assay of initiation by RNA polymerase II in yeast extracts. Initiation was enhanced by yeast and mammalian activator proteins. The adenoviral major late promoter fused to the G-minus sequence was transcribed in yeast extracts with an efficiency comparable to that observed in HeLa extracts, showing that promoters as well as transcription factors are functionally interchangeable across species. Initiation occurred at different sites, approximately 30 and 63 to 69 base pairs downstream of the TATA element of the adenoviral promoter in HeLa and yeast extracts, respectively, distances characteristic of initiation in the two systems in vivo. A component of the transcription system and not the promoter sequence determines the distance to the initiation site.

MANY ASPECTS OF TRANSCRIPTION by RNA polymerase II are conserved between yeast and higher eukaryotes. There is extensive amino acid sequence similarity among the largest subunits of the yeast, *Drosophila*, and mammalian polymerases (*1*). Other components of the transcription apparatus, such as TATA-binding and enhancer-binding factors, are functionally interchangeable between yeast and mammalian systems (*2–5*). There are, nonetheless, significant differences between the two systems. TATA elements are located from 40 to 120 or more base pairs upstream of the initiation site of a yeast promoter, and where these elements occur, they are absolutely required (*6*). By contrast, the distance between TATA elements and transcription start sites is smaller, 25 to 30 bp, and more uniform among mammalian promoters; in some cases, deletion of a TATA element does not reduce the frequency of initiation but rather impairs the precision of the process (*7*). It has been shown that if a yeast TATA-binding protein is substituted for the corresponding factor from HeLa cells, initiation still occurs in the manner characteristic of mammalian systems (*2, 3*). This implies that a factor other than the TATA-binding protein measures out the distance to the initiation site, or that the promoter sequence dictates the location of the site. Here we report on studies with a yeast transcription system that rule out the latter possibility and that may help identify the protein factor that locates the initiation site.

The recent development of a yeast RNA polymerase II transcription system (*8*) depended on hybridization with an RNA probe to reveal accurately initiated transcripts against a high background of nonspecific reaction. This method of detection is time-consuming and laborious. We tried a number of alternatives, such as run-off and primer extension assays, and had most success with an approach involving transcription of a template devoid of G residues (*9*). A form of the yeast *CYC1* promoter was

Department of Cell Biology, Beckman Laboratories, Fairchild Center, Stanford School of Medicine, Stanford, CA 94305.