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The Ubiquitous Transcription Factor Oct-1 and the Liver-Specific Factor HNF-1 Are Both Required to Activate Transcription of a Hepatitis B Virus Promoter

DAO-XIU ZHOU AND T. S. BENEDICT YEN*

Department of Pathology, University of California School of Medicine, San Francisco, California 94143-0506, and Anatomic Pathology Service 113B, Veterans Affairs Medical Center, 4150 Clement Street, San Francisco, California 94121

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The liver-specific transcription factor HNF-1 activates transcription of several mammalian hepatocyte-specific genes. The hepatitis B virus preS1 promoter shows hepatocyte specificity, which has been ascribed to binding of HNF-1 to a cognate DNA sequence upstream of the TATA box. We show here that there is an adjacent site that binds the ubiquitous transcription factor Oct-1. Both the Oct-1 and HNF-1 sites are necessary for liver-specific transcription of the preS1 promoter, but neither site alone activates transcription. The Oct-1 site is also necessary for activation of the preS1 promoter in HeLa cells expressing transfected HNF-1. Our results show that while Oct-1 is not restricted to hepatocytes, it nevertheless can play a critical role in the expression of a liver-specific gene.

The transcriptional activity of any given gene is governed by the constellation of *trans*-acting cellular factors that bind to its promoter and other *cis*-acting DNA elements (reviewed in references 29 and 31). The study of model viral genes, such as the simian virus 40 early gene, has been particularly fruitful in delineating these *cis*- and *trans*-acting factors in mammalian cells (reviewed in reference 23). Many of the viral genes studied are relatively non-cell type specific and have been mostly studied in dedifferentiated cells such as HeLa cells, hence, they have mostly given insights into non-cell-type-specific transcription. It is likely that analysis of promoters of viruses which show specific tissue tropism can give similarly useful information on cell-type-specific transcription.

Hepatitis B virus (HBV) is a hepatotropic virus with four known promoters, all on one strand of the DNA genome (reviewed in references 5 and 18). The two surface gene promoters are in tandem, approximately 400 bp apart (Fig. 1). Transcription from the upstream, TATA-containing promoter (preS1 or large surface antigen gene promoter) gives rise to an mRNA coding for the large surface antigen, while transcription from the downstream, TATA-less promoter (S or major surface antigen gene promoter) gives rise to several heterogeneous RNA species, coding for both the middle surface and major surface antigens (6, 8, 40, 41, 48, 50). All three forms of the surface antigen, which constitute the viral envelope proteins, are needed for virion assembly and maturation.

Transcription from the preS1 promoter shows hepatocyte specificity in transient transfection assays (8, 40). This cell type specificity is believed to account partially for the hepatotropism of HBV and has been attributed to the presence of an HNF-1 binding site approximately 75 bp upstream of the RNA start site (9, 11). HNF-1 (also called LF-B1, APF, and HP1) is a tissue-specific factor known to activate transcription of several liver-specific mRNAs, such as those for fibrinogen, albumin, α1-antitrypsin, and α-fetoprotein (7, 11, 12, 28, 32, 46). Its cDNA has been cloned (4, 15), and the deduced amino acid sequence places it in the homeodomain group of transcription factors (13).

In this report, we show that there is another *cis*-acting element immediately downstream of the HNF-1 site in the preS1 promoter. This element is required for HNF-1 to activate transcription of the preS1 promoter in both liver and HeLa cells, and it binds the ubiquitous transcription factor Oct-1 (OTF-1), another member of the homeodomain group of transcription factors (51).

MATERIALS AND METHODS

Plasmids and in vitro transcription and translation. All HBV sequences were derived from plasmid pHBV2, which contains two head-to-tail copies of HBV strain adw DNA, cloned by Valenzuela et al. (54). Plasmid pSB was made by using restriction enzymes *Ssp*I and *Eco*RI to excise the preS1 and S promoters (−132 to +421 relative to preS1 mRNA start site) and inserting the fragment between the *Sma*I and *Eco*RI sites of pUC18. The pSBΔ1, pSBΔ2, and pSBΔ1+2 mutants (Fig. 2B) were generated by oligonucleotide-directed mutagenesis (27) of the preS1 promoter fragment in pTZ19U by using a kit from Bio-Rad and were sequenced (44) with the Sequenase II kit (U.S. Biochemical).

The chlorampenicol acetyltransferase (CAT) expression plasmids were constructed by excising the wild-type or mutated preS1 promoter in the pSB series of plasmids with *Hin*dIII and *Bst*EII and placing it into the *Hin*dIII site of the promoterless CAT plasmid pSV0CAT (20), using blunt-end ligation. The orientation was determined by double restriction enzyme digestions. Plasmid pBB was constructed by digesting plasmid pHBV2, containing two head-to-tail copies of HBV DNA, with *Ssp*I and *Bgl*II and inserting the 2.5-kbp fragment, containing the surface and X genes, the enhancer, and the polyadenylation site (Fig. 1), into the *Bam*HI site of pUC18 with the aid of a *Bam*HI-*Ssp*I adapter. Clustered mutations in the preS1 promoter of pBB were introduced by

* Corresponding author.

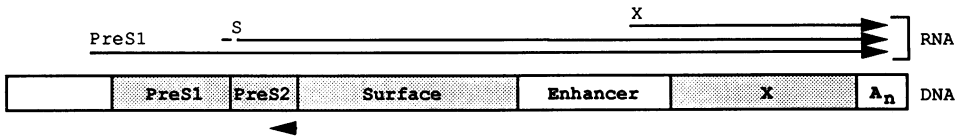

FIG. 1. Representation of the portion of the HBV genome containing the surface antigen and X genes and the transcripts coded by this region. Shaded areas are open reading frames; A_n is the polyadenylation site. The arrowhead shows the location of the oligonucleotide used for primer extension analysis in Fig. 7.

replacing the *Ssp*I-to-*Bst*EII fragment of pBB with the corresponding fragment of pSBΔ1, pSBΔ2, or pSBΔ1+2.

The expression plasmid pON-HNF contains the rat HNF-1 cDNA (15) under the control of the cytomegalovirus early promoter. It was constructed by digesting the HNF-1 cDNA plasmid pB1.4 (15; courtesy of R. Cortese) with *Asp*718 and *Bam*HI and inserting the fragment into the CMV expression plasmid pON260 (49), which had been digested with *Hin*dIII and *Pvu*II, with blunt-end ligation.

Plasmid pBSOct-1 (courtesy of W. Herr) contains the human Oct-1 cDNA under the control of the T7 promoter (51). RNA was synthesized in vitro by linearizing this plasmid with *Hin*dIII and incubating it with T7 polymerase and all four nucleoside triphosphates (43), as instructed by the manufacturer (Promega); it was used to program 35 μl of rabbit reticulocyte lysate as instructed by the manufacturer (Promega).

Plasmid pUC-multiocta (courtesy of T. Parslow) contains two copies of the octamer sequence of the immunoglobulin heavy-chain enhancer inserted into the *Bam*HI site of pUC18 (38).

Cell culture, transfection, CAT assay, and primer extension. All cells were grown at 37°C in DMEM (Dulbecco modified Eagle medium)-H21 with 10% fetal bovine serum under 6% CO_2. For CAT analysis, cells were transfected for 4 h with the calcium phosphate coprecipitation method (20) and harvested 46 h after the beginning of transfection. Cell extracts were obtained by freeze-thawing and assayed for CAT activity by incubation with acetyl coenzyme A and ^{14}C-chloramphenicol, followed by thin-layer chromatography (20). All CAT activities were normalized to the activity of cells transfected with pRSVCAT (20), with the CAT gene under the control of the Rous sarcoma viral promoter.

For primer extension, cells were transfected for 15 h with calcium phosphate and harvested 40 h after the beginning of transfection. RNA was harvested by guanidine lysis and centrifugation through CsCl (10). In each reaction, 5 μg of total RNA was incubated with 5 U of Avian myeloblastosis virus reverse transcriptase (Bethesda Research Laboratories) and 1 ng of primer labeled at the 5′ end by [γ-^{32}P]ATP and polynucleotide kinase (43). After 1 h at 42°C, the mixture was electrophoresed on a denaturing 6% acrylamide gel, and the extended products were visualized by autoradiography (43). The primer used has the sequence 5′-AGAGGCAA TATTCGGAGCAGGGTTTAC and should give products of 521 bases for the preS1 transcript and of 135 to 136, 115 to 116, and 105 bases for the S transcript (Fig. 1).

DNase I protection and gel shift assays. Nuclear extracts were obtained as described by Osborn et al. (37). For DNase I protection analysis (24), plasmid pSB was digested with *Acc*I and *Bst*EII and incubated with [α-^{32}P]dCTP or [α-^{32}P]dGTP and Klenow fragment of DNA polymerase I (43) to label the lower and upper strands, respectively. A 10-ng sample of each labeled fragment was subjected to chemical scission (30), or digestion with 40 ng DNase I at room temperature, in the presence of 30 μg of bovine serum albumin or HepG2 cell extract protein.

For gel shift analysis (16), 1 ng of DNA, labeled as described above, was incubated with 2 μg of poly(dI-dC) and 5 μg of nuclear extract protein for 30 min at room temperature. In some experiments, instead of nuclear extracts, 1 μl of reticulocyte lysate (Promega), before or after programming by Oct-1 RNA, was used. For competition, a 50-fold molar excess of unlabeled DNA was included in the mixture. After incubation, the samples were electrophoresed at 140 V in a 6% acrylamide gel at room temperature, and the bands were visualized by autoradiography. In addition, in some experiments 1 μl of rabbit antibodies against Oct-1 and HNF-1, kind gifts of W. Herr (Cold Spring Harbor Laboratory) and G. Crabtree (Stanford University), respectively, was included in the mixture. Pooled nonimmune rabbit serum (GIBCO) was used as a negative control.

RESULTS

Identification of an Oct-1 site in the preS1 promoter. HepG2 hepatoblastoma cells (1) contain HNF-1 and allow efficient transcription of the preS1 promoter of HBV (9). When HepG2 extracts were used in DNase I protection assay of the preS1 promoter, a footprint covering the previously described HNF-1 site was observed on both strands (site 2; Fig. 2A). However, the footprint extended at least 20 bp downstream of the known HNF-1 site (9, 11), and a nuclease-hypersensitive site was present between this portion of the footprint and the HNF-1 site on the lower strand (Fig. 2A). These data suggested that another factor was binding to this downstream site (site 1; Fig. 2A).

To confirm this observation, HepG2 cell extracts were used in gel shift experiments with the preS1 promoter fragment. Three shifted bands were observed (Fig. 3), presumably corresponding to DNA with factors bound to site 1, site 2, or both sites. To assign the bands to the corresponding occupied sites, we mutated the preS1 promoter fragment (SB) within either site 1 (SBΔ1) or site 2 (SBΔ2) (Fig. 2B). Shifted bands 2 and 3 were competed for by the preS1 promoter with mutated site 1, while bands 1 and 3 were competed for by the promoter with mutated site 2 (Fig. 3). All three bands were competed for by the wild-type promoter. These results showed that band 1 corresponded to DNA with site 1 occupied, band 2 corresponded to DNA with site 2 (the HNF-1 site) occupied, while band 3 corresponded to DNA with both sites occupied.

Examination of site 1 revealed the sequence ATTTaCAT in the top strand (Fig. 1), almost identical to the consensus octamer site ATTTGCAT (reviewed in reference 25). This similarity raised the possibility that site 1 binds the ubiquitous transcription factor Oct-1 (51). This was confirmed by four independent observations. First, band 1 but not band 2 in the HepG2 gel shift assay was competed for by excess DNA with authentic octamer sites from the immunoglobulin

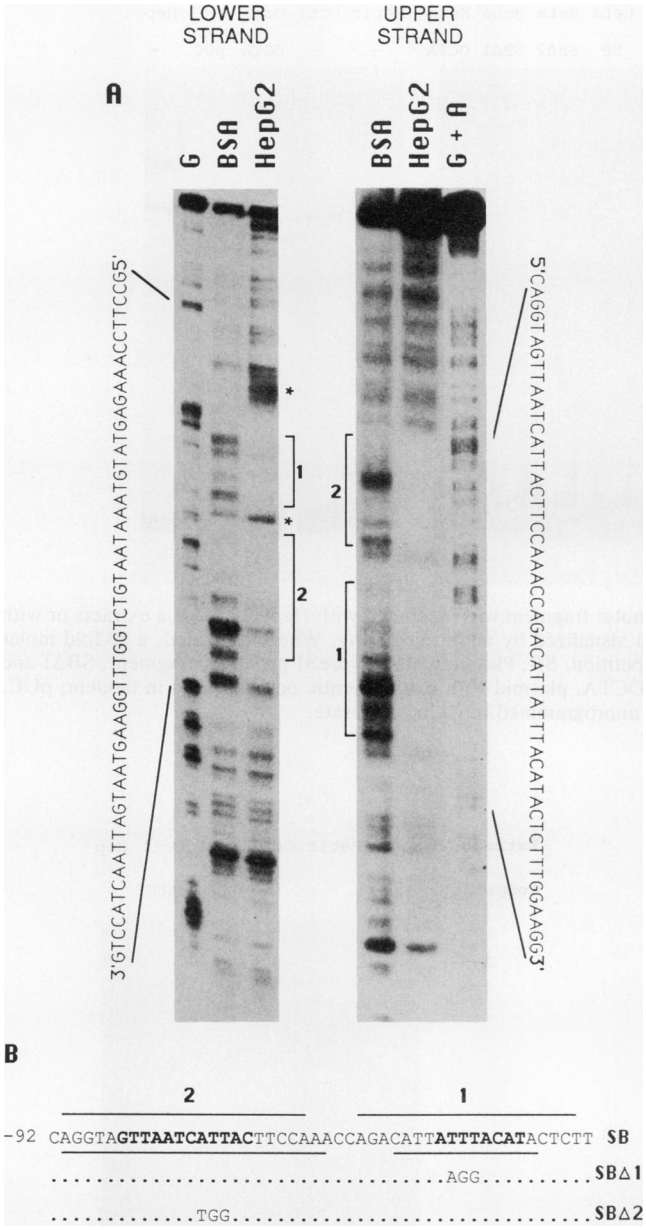

FIG. 2. DNase I protection analysis of the preS1 promoter. (A) A fragment of HBV DNA with the preS1 promoter was labeled at the 3′ end of the lower or upper stand, incubated with bovine serum albumin (BSA) or HepG2 hepatoblastoma extracts, digested with DNase I, electrophoresed on a denaturing gel, and visualized by autoradiography. The brackets indicate areas of protection deduced from several independent assays; asterisks indicate hypersensitive sites only in the region of interest. The G and G + A lanes show partial sequences (cleaved at G or G + A residues, respectively) for alignment. (B) Summary of the protection data. Overlines indicated protected areas in the upper strand; underlines indicate protected areas in the lower strand. SB is the wild-type preS1 promoter sequence; SBΔ1 and SBΔ2 are mutated promoters generated by in vitro mutagenesis, used in gel shift and transcriptional analyses (Fig. 3 and 6 to 8).

heavy-chain enhancer (Fig. 3). Second, the preS1 promoter in the presence of HeLa cell extracts (known to contain Oct-1) gave rise to a single shifted band which comigrated with band 1 of HepG2 extracts, and this band showed competition patterns identical to those of band 1 (Fig. 3). Third, the preS1 promoter in the presence of Oct-1 synthesized by in vitro transcription-translation gave rise to a shifted band with an electrophoretic mobility similar to that of band 1 (Fig. 3). Fourth, the preS1 promoter can compete for Oct-1 binding to a labeled DNA fragment with an authentic octamer site (Fig. 4).

The above conclusion was strengthened by gel shift analysis in the presence of specific antibodies against Oct-1 and HNF-1 (courtesy of W. Herr and G. Crabtree, respectively). The Oct-1 antibodies, which completely block DNA binding (21a), specifically inhibited the formation of shifted bands 1 and 3 (Fig. 5), showing that site 1 was occupied by authentic Oct-1. On the other hand, the HNF-1 antibodies, which only partially block DNA binding (11a), inhibited the formation of shifted band 2, while band 3 was replaced by a band more diffuse and slightly slower in mobility (band 3′; Fig. 5). Most likely, this new band represents the supershift, i.e., a complex containing both HNF-1 and anti-HNF-1 antibodies bound to the DNA. It is worth noting that the antibodies to HNF-1 are specific (11a) and do not recognize related factors such as vHNF, a transcriptionally inactive factor that also binds to HNF-1 sites (3). Therefore, the factor that is bound to site 2 in the preS1 promoter must be HNF-1.

The Oct-1 site is necessary for HNF-1 to activate the preS1 promoter in both liver and HeLa cells. Oct-1 is present in all adult cell types and activates transcription of several housekeeping genes, such as those for histone 2B and small nuclear RNAs (2, 14, 21, 33, 42, 52). However, in vivo it is incapable of activating many other genes with octamer sites, despite binding to these sites in vitro (17, 45, 53; reviewed in reference 25). To determine whether the octamer site in the preS1 promoter is important for transcriptional activation, we performed transient transfection assays of CAT reporter plasmids. The preS1 promoter (SB) directed the synthesis of a high level of CAT activity in HepG2 hepatoblastoma cells (Fig. 6). Mutations in either the Oct-1 site (SBΔ1) or the HNF-1 site (SBΔ2) reduced the CAT activity by >10-fold; however, no further significant reduction was observed with the double mutant (SBΔ1+2) (Fig. 6). Similar results were obtained with HuH-7 cells (data not shown), another well-differentiated hepatoma line (34) that contains HNF-1 (9). In contrast, the preS1 promoter was weak in HeLa cells, and mutations in either site did not significantly change its activity (Fig. 6). Therefore, both the Oct-1 and HNF-1 sites are necessary for liver-specific transcription of the preS1 promoter, and they cannot act independently of each other. The most likely explanation is that the ubiquitous factor Oct-1 cooperates with the hepatocyte-specific factor HNF-1 to activate the preS1 promoter.

Our results thus far do not rule out the possibility that there is a hepatocyte-specific modification of Oct-1 that is responsible for the activity of the Oct-1 site in liver cells. We therefore cotransfected the CAT gene driven by the preS1 promoter with a plasmid expressing rat HNF-1 into HeLa cells. The preS1 promoter (SB) was >18-fold more active in HeLa cells with transfected HNF-1 than in HeLa cells without HNF-1 (Fig. 7). This activation was again dependent on both the octamer and HNF-1 sites, since mutation of either site (SBΔ1 and SBΔ2, respectively) rendered the promoter essentially unresponsive to transfected HNF-1 (Fig. 7). These results confirmed that the octamer-binding cofactor is also present in HeLa cells and hence cannot be a hepatocyte-specific form of the ubiquitous factor Oct-1.

The Oct-1 site is active in the intact surface gene. In the HBV genome, downstream of the surface gene there are an

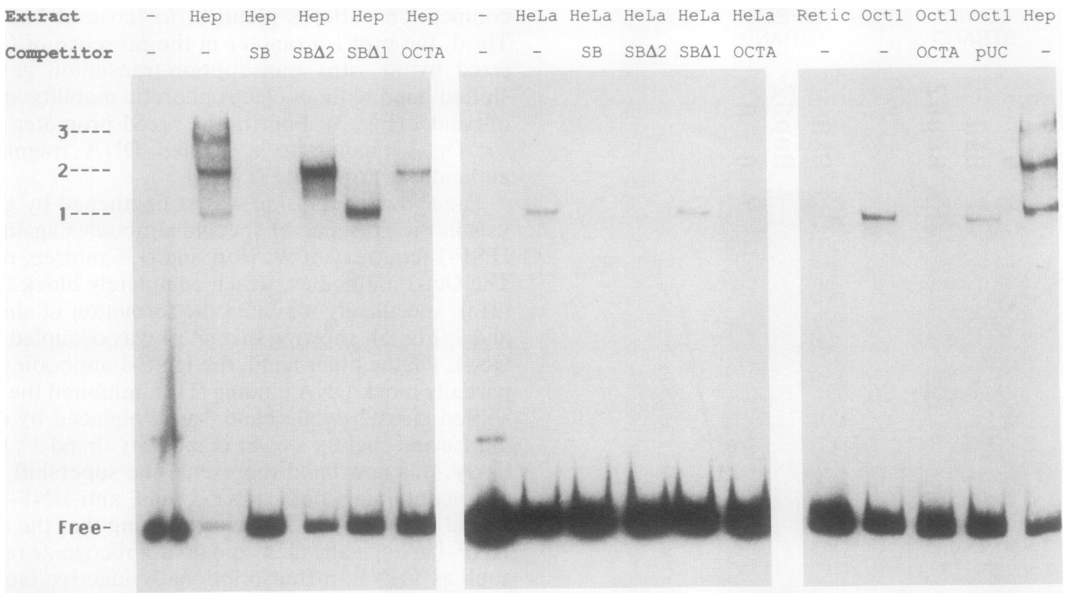

FIG. 3. Gel shift analysis of the preS1 promoter. A labeled preS1 promoter fragment was incubated with HepG2 or HeLa extracts or with in vitro-synthesized Oct-1, electrophoresed on a nondenaturing gel, and visualized by autoradiography. Where indicated, a 50-fold molar excess of unlabeled DNA was present in the incubation mixture for competition. SB, Plasmid with the preS1 promoter fragment; SBΔ1 and SBΔ2, plasmids with mutated preS1 promoter fragments (see Fig. 2B); OCTA, plasmid with two authentic octamer sites in tandem; pUC, pUC18; Oct-1, reticulocyte lysate programmed with Oct-1 RNA; Retic, unprogrammed reticulocyte lysate.

enhancer (8, 39, 47, 55) and the X gene (Fig. 1), which codes for a transcriptional transactivator (reviewed in reference 56). It seemed possible that these confounding factors influence the activity of the HNF-1 and Oct-1 sites in the preS1 promoter. In addition, only ~400 bp downstream of the preS1 promoter is the major surface gene (S) promoter, which therefore may also be under the control of these two *cis*-acting elements. To examine these possibilities, we measured the amount of preS1 and S transcripts in hepatoma cells transiently transfected with a plasmid containing a fragment of HBV DNA with the surface and X genes under the control of their native promoters and enhancer (Fig. 1). As expected from previous work (6, 41, 48, 50), a much larger amount of S transcript than preS1 transcript was present (Fig. 8, lane BB). Mutation of either the Oct-1 or HNF-1 site (lanes BBΔ1 and BBΔ2, respectively) dramatically reduced the preS1 transcript level, while mutation of both sites (lane BBΔ1+2) did not have a significant additional effect. In contrast, none of the mutations had a significant effect on the level of the S transcript. These results confirmed our previous results using CAT plasmids and showed that neither the enhancer nor the X gene product modulated the effect of the Oct-1 site on the preS1 promoter. Furthermore, neither the Oct-1 nor the HNF-1 site had an effect on the downstream S promoter, which, unlike the preS1 promoter, does not have a TATA box.

DISCUSSION

We have confirmed that an HNF-1 site in the HBV genome is important for hepatocyte-specific activity of the preS1 promoter but not of the S promoter. Since the HNF-1 site is much closer to the preS1 promoter than the S promoter, this differential regulation may be due solely to a distance effect. Alternatively, since the preS1 promoter contains a TATA box while the S promoter does not, it is

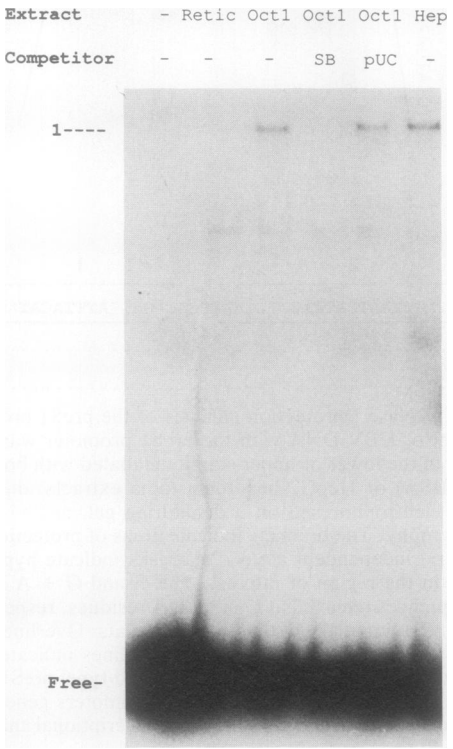

FIG. 4. Gel shift analysis of an authentic Oct-1 site. A labeled fragment of DNA with an authentic octamer site from the immunoglobulin heavy-chain enhancer, with or without a 50-fold excess of competing unlabeled DNA, was incubated with unprogrammed reticulocyte lysate (Retic), in vitro-synthesized Oct-1, or HepG2 cell lysate and electrophoresed on a nondenaturing gel. SB, Plasmid with the preS1 promoter; pUC, pUC18.

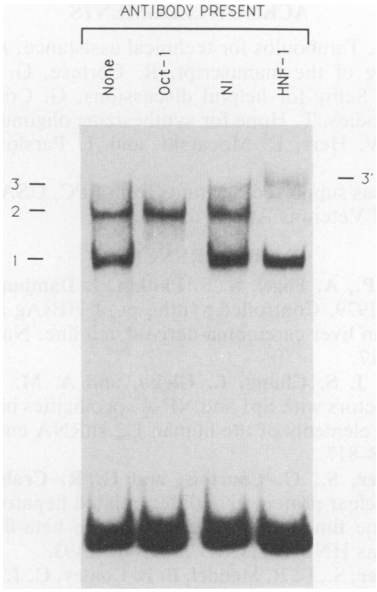

FIG. 5. Gel shift analysis in the presence of specific antibodies. The labeled preS1 promoter fragment was incubated with 0.5 μl of HepG2 cell extracts, with or without specific rabbit antibodies against Oct-1 and HNF-1, or with nonimmune rabbit serum (NI).

possible that HNF-1 can activate only via the TATA-binding factor.

More interestingly, our results further indicate that the tissue-specific transcription factor HNF-1 is incapable of activating transcription of the HBV preS1 promoter by itself. Rather, it is dependent on an adjacent site being occupied by a factor that is not restricted to hepatocytes. This other factor has all the characteristics of the well-known transcription factor Oct-1: it binds to an octamer site, it is not cell type specific, it is bound by anti-Oct-1 antibodies, and it cannot activate transcription by itself. Furthermore, our gel shift analysis reveals only one octamer-binding factor in HeLa and HepG2 cells, with the same electrophoretic mobility as authentic Oct-1 (Fig. 3 and 4). Therefore, we believe that Oct-1 is the cofactor necessary for HNF-1 to activate the preS1 promoter.

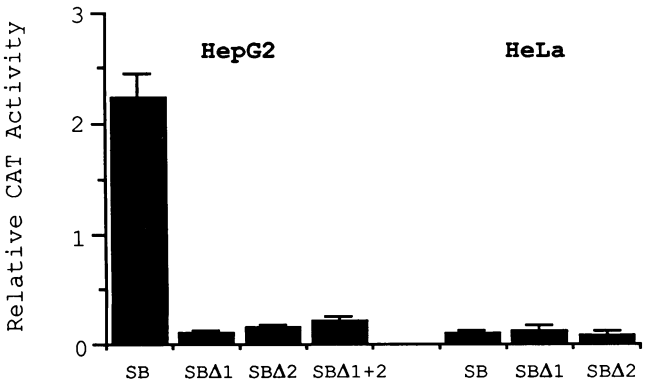

FIG. 6. Role of the Oct-1 and HNF-1 sites in transcription from the preS1 promoter. CAT expression plasmids driven by the preS1 promoter (10 μg per plate), either wild type (SB) or with mutations in the Oct-1 (SBΔ1) or HNF-1 (SBΔ2) site or both sites (SBΔ1+2), were transfected into HepG2 hepatoblastoma or HeLa cells, and the CAT activity was measured after 2 days. The results represent means ± standard deviations of three independent transfections and are normalized to the activity of pRSVCAT (set at 100).

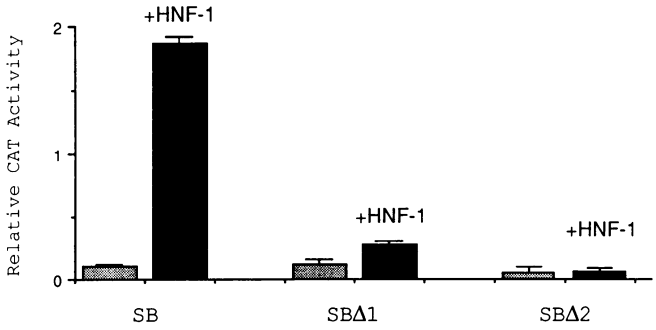

FIG. 7. Effect of HNF-1 on transcription from wild-type and mutant preS1 promoters in HeLa cells. The preS1 promoter-driven CAT plasmids (3.3 μg per plate) were cotransfected into HeLa cells with either pUC118 or a plasmid expressing rat HNF-1 (6.7 μg per plate), and the CAT activity was measured after 2 days. The results represent the means ± standard deviations of three independent transfections and are normalized to the activity of the pRSVCAT (set at 100).

These findings are unexpected in that Oct-1 has so far been implicated only in transcription of housekeeping genes. However, even for at least some of the housekeeping genes, Oct-1 is incapable of activating transcription in the absence of other ubiquitous factors that bind to other *cis* elements in these promoters (2, 33, 42). The transcription factor Sp1 has been implicated as one of these factors (2), but the other factors are as yet uncharacterized. Similarly, the herpes simplex virus transactivator VP16 (reviewed in reference 19) depends on both Oct-1 bound to an octamer site and another cellular factor bound to an adjacent site (26). Furthermore, HNF-1 activation of the α1-antitrypsin promoter is known to be entirely dependent on another factor that binds immediately upstream of the HNF-1 site (32). While this other factor is also transcriptionally incompetent by itself, it is not Oct-1, since it is liver specific (32).

Therefore, Oct-1 and HNF-1 appear to activate transcription only in combination with each other or with other, as yet unidentified factors. It has been noted by others that certain enhancer elements are inactive unless combined with other elements (17, 36). In addition, the yeast cell-type-specific factor α2 activates transcription only in concert with the ubiquitous factor MCM1 (reviewed in reference 22). Our results show that a similar situation can be seen with upstream promoter elements implicated in mammalian cell-type-specific transcription. A promoter constructed from elements that function cooperatively would have obvious functional advantages during development in that it allows a limited number of tissue-specific factors to generate a greater variety of different specificities. While fewer different cell type specificities would be generated if one of these factors were ubiquitous, this would also provide a simple means of expressing a gene in a select subset of cell types that otherwise have little in common. For example, HNF-1 is also expressed in the kidney (4), and indeed, HBV is known to infect renal epithelial cells (5).

The mechanism by which Oct-1 and HNF-1 are both needed to activate transcription is not clear. However, in contrast to the case with the yeast α2 factor and MCM1 (reviewed in reference 22), it does not appear to be through cooperative binding to DNA, since gel shift analysis with

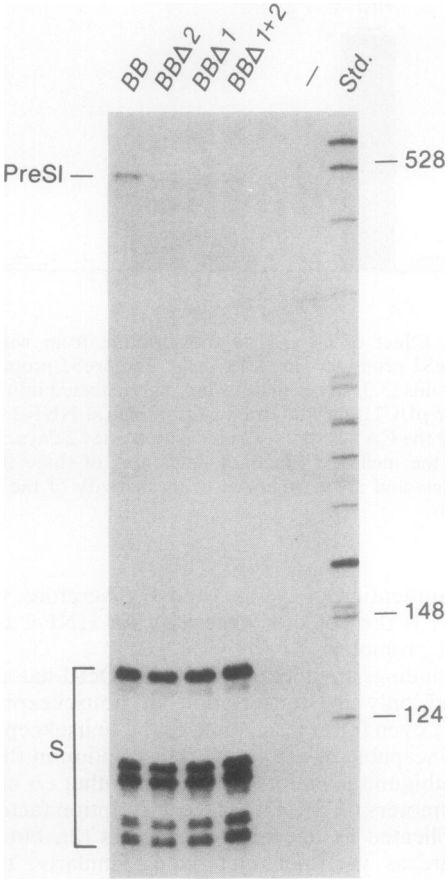

FIG. 8. Role of the Oct-1 and HNF-1 sites for preS1 transcription in the presence of the HBV enhancer and X protein. A series of plasmids containing a fragment (nucleotides 2669 to 1987) of HBV DNA, including the entire surface and X genes, the enhancer, and the polyadenylation site (see Fig. 1), was constructed. A 10-μg amount of each plasmid was transfected into HuH-7 hepatoma cells. Total RNA was harvested after 2 days and subjected to primer extension analysis of the amount of RNA transcribed from the preS1 or S promoter. Lanes: BB, wild-type HBV; BBΔ1 and BBΔ2, the same clustered mutations introduced into the preS1 promoter as in Fig. 2B; −, a control reaction with RNA from untransfected cells; Std., pBR322 DNA digested with *Msp*I and labeled with [α-^{32}P]dCTP and Klenow enzyme. The numbers represent the sizes in bases of selected marker bands.

HepG2 extracts reveals large amounts of preS1 promoter with only Oct-1 or HNF-1 bound (Fig. 3), and competition with excess DNA with only one site does not appear to affect binding to the other site (Fig. 3). One possible hint as to the mechanism comes from the finding of Chang et al. (9) that the HNF-1 site alone can partially activate the preS1 promoter if it is moved to within 5 bp of the TATA box. We speculate that HNF-1 carries a transcriptional activation domain but binds only weakly to the transcription initiation complex. Oct-1 would act to stabilize this interaction. A similar situation has been described for the yeast transcription factor Gal4: it is active by itself when its binding site is close to the TATA box, but it depends on another factor, Gal11, when its binding site is further upstream (35). Further studies will be needed to confirm our hypothesis.

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REFERENCES

1. **Aden, D. P., A. Fogel, S. S. Plotkin, I. Damjanov, and B. B. Knowles.** 1979. Controlled synthesis of HBsAg in a differentiated human liver carcinoma-derived cell line. Nature (London) **282**:615–617.
2. **Ares, M., J. S. Chung, L. Giglio, and A. M. Weiner.** 1987. Distinct factors with Sp1 and NF-A specificities bind to adjacent functional elements of the human U2 snRNA enhancer. Genes Dev. **1**:808–817.
3. **Baumhueter, S., G. Courtois, and G. R. Crabtree.** 1988. A variant nuclear protein in dedifferentiated hepatoma cells binds to the same functional sequences in the beta-fibrinogen gene promoter as HNF-1. EMBO J. **7**:2485–2493.
4. **Baumhueter, S., D. B. Mendel, P. B. Conley, C. J. Kuo, C. Turk, M. K. Graves, C. A. Edwards, G. Courtois, and G. R. Crabtree.** 1990. HNF-1 shares three sequence motifs with the POU domain proteins and is identical to LF-B1 and APF. Genes Dev. **4**:372–379.
5. **Blum, H. E., W. Gerok, and G. N. Vyas.** 1989. The molecular biology of hepatitis B virus. Trends Genet. **5**:154–158.
6. **Cattaneo, R., H. Will, N. Hernandez, and H. Schaller.** 1983. Signals regulating hepatitis B surface antigen transcription. Nature (London) **305**:336–338.
7. **Cereghini, S., M. Raymondjean, A. G. Carranca, P. Herbomel, and M. Yaniv.** 1987. Factors involved in control of tissue-specific expression of albumin gene. Cell **50**:627–638.
8. **Chang, H. K., and L. P. Ting.** 1989. The surface gene promoter of the human hepatitis B virus displays a preference for differentiated hepatocytes. Virology **170**:176–183.
9. **Chang, H. K., B. Y. Wang, C. H. Yuh, C. L. Wei, and L. P. Ting.** 1989. A liver-specific nuclear factor interacts with the promoter region of the large surface protein gene of human hepatitis B virus. Mol. Cell. Biol. **9**:5189–5197.
10. **Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active RNA from sources enriched in ribonucleases. Biochemistry **18**:5294–5299.
11. **Courtois, G., S. Baumhueter, and G. R. Crabtree.** 1988. Purified hepatocyte nuclear factor 1 interacts with a family of hepatocyte-specific promoters. Proc. Natl. Acad. Sci. USA **85**:7937–7941.

11a. **Crabtree, G.** Personal communication.

12. **Feuerman, M. H., R. Godbout, R. S. Ingram, and S. M. Tilghman.** 1989. Tissue-specific transcription of the mouse α-fetoprotein gene promoter is dependent on HNF-1. Mol. Cell. Biol. **9**:4204–4212.
13. **Finney, M.** 1990. The homeodomain of the transcription factor LF-B1 has a 21 amino acid loop between helix 2 and helix 3. Cell **60**:5–6.
14. **Fletcher, C., N. Heintz, and R. G. Roeder.** 1987. Purification and characterization of OTF-1, a transcription factor regulating cell cycle expression of a human histone H2b gene. Cell **51**:773–781.
15. **Frain, M., G. Swart, P. Monaci, A. Nicosia, S. Stämpfli, R. Frank, and R. Cortese.** 1989. The liver-specific transcription factor LF-B1 contains a highly diverged homeobox DNA binding domain. Cell **59**:145–157.
16. **Fried, M., and D. Crothers.** 1981. Equilibria and kinetics of lac repressor-operator interactions by polyacrylamide gel electrophoresis. Nucleic Acids Res. **9**:6505–6525.
17. **Fromental, C., M. Kanno, H. Nomiyama, and P. Chambon.** 1988. Cooperativity and hierarchical levels of function organization in the SV40 enhancer. Cell **54**:943–953.
18. **Ganem, D., and H. E. Varmus.** 1987. The molecular biology of the hepatitis B viruses. Annu. Rev. Biochem. **56**:651–693.

19. **Goding, C. R., and P. O'Hare.** 1989. Herpes simplex virus Vmw65-octamer binding protein interaction: a paradigm for combinatorial control of transcription. Virology **173**:363–367.
20. **Gorman, C.** 1985. High efficiency gene transfer into mammalian cells, p. 143–190. *In* D. M. Glover (ed.), DNA cloning vol. II. IRL Press, Oxford.
21. **Harvey, R. P., A. J. Robins, and J. R. E. Wells.** 1982. Independently evolving chicken histone H2B genes: identification of a ubiquitous H2B-specific 5′ element. Nucleic Acids Res. **10**: 7851–7863.
21a. **Herr, W.** Personal communication.
22. **Herskowitz, I.** 1989. A regulatory hierarchy for cell specialization in yeast. Nature (London) **342**:749–757.
23. **Jones, N. C., P. W. J. Rigby, and E. B. Ziff.** 1988. Trans-acting protein factors and the regulation of eucaryotic transcription: lessons from studies on DNA tumor viruses. Genes Dev. **2**:267–281.
24. **Kadonaga, J. T., and R. Tjian.** 1986. Affinity purification of sequence-specific DNA binding proteins. Proc. Natl. Acad. Sci. USA **83**:5889–5893.
25. **Kemler, I., and W. Schaffner.** 1990. Octamer transcription factors and the cell-type specificity of immunoglobulin gene expression. FASEB J. **4**:1444–1449.
26. **Kristie, T. M., J. H. LeBowitz, and P. A. Sharp.** 1989. The octamer-binding proteins form multi-protein-DNA complexes with the herpes simplex virus αTIF regulatory protein. EMBO J. **8**:4229–4238.
27. **Kunkel, T. K.** 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82**:488–491.
28. **Lichtensteiner, S., and U. Schibler.** 1989. A glycosylated liver-specific transcription factor stimulates transcription of the albumin gene. Cell **57**:1179–1187.
29. **Maniatis, T., S. Goodbourn, and J. A. Fisher.** 1987. Regulation of inducible and tissue-specific gene expression. Science **236**: 1237–1245.
30. **Maxam, A. M., and W. Gilbert.** 1977. A new method for sequencing DNA. Proc. Natl. Acad. Sci. USA **74**:560–564.
31. **Mitchell, P. J., and R. Tjian.** 1989. Transcription regulation in mammalian cells by sequence-specific DNA binding proteins. Science **245**:371–378.
32. **Monaci, P., A. Nicosia, and R. Cortese.** 1988. Two different liver-specific factors stimulate in vitro transcription from the human α1-antitrypsin promoter. EMBO J. **7**:2075–2087.
33. **Murphy, S., A. Pierani, C. Scheidereit, M. Melli, and R. G. Roeder.** 1989. Purified octamer binding transcription factors stimulate RNA polymerase III-mediated transcription of the 7SK RNA gene. Cell **59**:1071–1080.
34. **Nakabayashi, H., K. Taketa, M. Miyano, T. Yamane, and J. Sato.** 1982. Growth of human hepatoma cell lines with differentiated functions in chemically defined medium. Cancer Res. **42**:3858–3863.
35. **Nishizawa, M., Y. Suzuki, N. Yasuhisa, M. Kunihiro, and T. Fukasawa.** 1990. Yeast Gal11 protein mediates the transcriptional activation signal of two different transacting factors, Gal4 and general regulatory factor I/repressor/activator site binding protein I/translation upstream factor. Proc. Natl. Acad. Sci. USA **87**:5373–5377.
36. **Ondek, B., L. Gloss, and W. Herr.** 1988. The SV40 enhancer contains two distinct levels of organization. Nature (London) **333**:40–45.
37. **Osborn, L., S. Kunkel, and G. J. Nabel.** 1989. Tumor necrosis factor α and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor kappa B. Proc. Natl. Acad. Sci. USA **86**:2336–2340.
38. **Parslow, T. G., S. D. Jones, B. Bond, and K. R. Yamamoto.** 1987. The immunoglobulin octanucleotide: independent activity and selective interaction with enhancers. Science **235**:1498–1501.
39. **Patel, N. U., S. Jameel, H. Isom, and A. Siddiqui.** 1989. Interactions between nuclear factors and the hepatitis B virus enhancer. J. Virol. **63**:5293–5301.
40. **Raney, A. K., D. R. Milich, A. J. Eason, and A. McLachlan.** 1990. Differentiation-specific transcriptional regulation of the hepatitis B virus large surface antigen gene in human hepatoma cell lines. J. Virol. **64**:2360–2368.
41. **Raul, L. B., D. N. Standring, O. Laub, and W. J. Rutter.** 1983. Transcription of hepatitis B virus by RNA polymerase II. Mol. Cell. Biol. **3**:1766–1773.
42. **Roebuck, K. A., D. P. Szeto, K. P. Green, Q. N. Fan, and W. E. Stumph.** 1990. Octamer and SPH motifs in the U1 enhancer cooperative to activate U1 RNA gene expression. Mol. Cell. Biol. **10**:341–352.
43. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
44. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
45. **Schirm, S., J. Jiricny, and W. Schaffner.** 1987. The SV40 enhancer can be dissected into multiple segments, each with a different cell type specificity. Genes Dev. **1**:65–72.
46. **Schorpp, M., W. Kugler, U. Wagner, and G. U. Ryffel.** 1988. Hepatocyte-specific promoter element HP1 of the Xenopus albumin gene interacts with transcriptional factors of mammalian hepatocytes. J. Mol. Biol. **202**:307–320.
47. **Shaul, Y., W. J. Rutter, and O. Laub.** 1985. A human hepatitis B viral enhancer element. EMBO J. **4**:427–430.
48. **Siddiqui, A., S. Jameel, and J. Mapoles.** 1986. Transcriptional control elements of hepatitis B surface antigen gene. Proc. Natl. Acad. Sci. USA **83**:566–570.
49. **Spaete, R. R., and E. S. Mocarski.** 1985. Regulation of cytomegalovirus gene expression: α and β promoters are *trans* activated by viral functions in permissive human fibroblasts. J. Virol. **56**:817–824.
50. **Standring, D. N., W. J. Rutter, H. E. Varmus, and D. Ganem.** 1984. Transcription of the hepatitis B surface antigen gene in cultured murine cells initiates within the presurface region. J. Virol. **50**:563–571.
51. **Sturm, R. A., G. Das, and W. Herr.** 1988. The ubiquitous octamer-binding protein Oct-1 contains a POU domain with a homeo box subdomain. Genes Dev. **2**:1582–1599.
52. **Tanaka, M., U. Grossniklaus, W. Herr, and N. Hernandez.** 1988. Activation of the U2 snRNA promoter by the octamer motif defines a new class of RNA polymerase II enhancer elements. Genes Dev. **2**:1764–1778.
53. **Tanaka, M., and W. Herr.** 1990. Differential transcription activation by Oct-1 and Oct-2: interdependent activation domains induce Oct-2 phosphorylation. Cell **60**:375–386.
54. **Valenzuela, P., M. Quiroga, J. Zaldivar, P. Gray, and W. J. Rutter.** 1980. The nucleotide sequence of the hepatitis B viral genome and the identification of the major viral genes. p. 57–70. *In* B. Fields, R. Jaenisch, and C. F. Fox (ed.), Animal virus genetics. Academic Press, Inc., New York.
55. **Vannice, J. L., and A. D. Levinson.** 1988. Properties of the hepatitis B virus enhancer: position effects and cell type nonspecificity. J. Virol. **62**:1305–1313.
56. **Yen, T. S. B., P. J. Mitchell, and E. Seto.** Mechanism of transcriptional trans-activation by the hepatitis B virus X protein, in press. *In* F. B. Hollinger, S. M. Lemon, and H. S. Margolis (ed.), Viral hepatitis—1990, Williams and Wilkins, Baltimore.