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Promoter-Specific Transactivation of Hepatitis B Virus Transcription by a Glutamine- and Proline-Rich Domain of Hepatocyte Nuclear Factor 1†

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The cloned transcription factor hepatocyte nuclear factor 1 (HNF1) transactivates transcription from the hepatitis B virus (HBV) large surface antigen promoter but does not influence the transcriptional activities of the other three HBV promoters. This indicates that this transcription factor can differentially influence the activities of the HBV promoters. By using a transient-transfection system, the major domain of the HNF1 polypeptide involved in transcriptional activation of the large surface antigen promoter in the human hepatoma cell line HepG2.1 has been mapped to a region that is rich in glutamine and proline residues (9 of 18) and is different from the previously identified regions of this factor responsible for in vitro transcriptional activation of a promoter containing human albumin promoter HNF1 binding sites. The human albumin promoter HNF1 binding site mediates transcriptional activation through the same HNF1 polypeptide domain as the HBV large surface antigen promoter HNF1 binding site in transient-transfection assays with HepG2.1 cells, suggesting that HNF1 may possess multiple transcriptional activation domains.

The hepatitis B virus (HBV) genome is a 3.2-kilobase (kb), partially double-stranded circular DNA molecule which, during infection, encodes transcripts of 3.5, 2.4, 2.1, and possibly 0.7 kb (6, 7, 30, 33, 54, 61). These transcripts are translated into the nucleocapsid and polymerase polypeptides, the large surface antigen polypeptide, the middle and major surface antigen polypeptides, and the X gene polypeptide, respectively (24). The regulatory sequence elements that control the expression of the HBV transcripts have been characterized, and a transcriptional enhancer element has been located between the 3′ end of the surface antigen gene and the 5′ end of the X gene (1, 5, 18, 19, 23, 31, 51, 55, 58). In addition, promoter sequences have been identified near the transcription initiation sites of each of the HBV RNAs (10, 15, 29, 34, 40, 42, 44, 45, 50, 52, 53, 56).

Characterization of the large surface antigen promoter has revealed the presence of a binding site for the liver-specific transcription factor hepatocyte nuclear factor 1 (HNF1; also referred to as LFB1, APF, and HP1), which is essential for high levels of transcription from this promoter in differentiated hepatoma cell lines (10, 40, 44). The binding site for HNF1 has been identified in the promoters of several genes and has been implicated in controlling their liver-specific expression (8, 9, 13, 14, 20, 26, 35, 36, 47, 57, 59). The cloning of the HNF1 cDNA (4, 11, 21) has permitted the tissue-specific expression of the HNF1 transcript to be determined. This analysis revealed that the HNF1 RNAs were predominantly expressed in liver (4, 21) and possibly kidney (4), which may explain why genes regulated by promoters containing HNF1 binding sites display a liver-specific pattern of expression.

The cloning of the HNF1 cDNA also permitted the functional characterization of the HNF1 polypeptide (11, 41). This analysis showed that the HNF1 polypeptide can be subdivided into several functionally distinct regions (11, 41). The amino-terminal 31 amino acids constitute the dimerization domain of the HNF1 polypeptide, and the amino-terminal 281 amino acids are required for sequence-specific DNA binding (11, 41). The DNA-binding domain contains, in addition to the dimerization domain, a region of homology to subregion A of the POU domain present in the Pit1/GHF, Oct-1, Oct-2, and Unc-86 transcription factors and a homeoboxlike domain (4, 21, 28). These regions are essential for binding of the HNF1 polypeptide to the HNF1 recognition sequence (11, 41). In addition, two transcriptional activation domains located between amino acids 281 and 318 and amino acids 547 and 628 of the HNF1 polypeptide were shown to influence the in vitro transcriptional activity of a promoter containing human albumin HNF1 binding sites in spleen nuclear extracts supplemented with HeLa cell-derived HNF1 polypeptides (41).

In the present analysis, the influence of expression of the cloned rat HNF1 cDNA (21) in transient-transfection assays on the transcriptional activities of the HBV promoters was examined in a dedifferentiated human hepatoma cell line, HepG2.1, which does not express levels of the HNF1 polypeptide capable of transactivating the large surface antigen promoter (44). This analysis demonstrated that the large surface antigen promoter was transcriptionally activated by exogenously expressed HNF1, whereas other HBV promoter activities were not affected by this transcription factor. It was also shown that the HNF1 polypeptide could transactivate a minimal promoter construct through either the large surface antigen promoter HNF1 binding site or the human albumin promoter HNF1 binding site. The transcriptional activation domain of the HNF1 polypeptide mediating this effect in HepG2.1 cells was located between amino acids 393 and 548, which is distinct from the transcriptional activation domain previously determined by in vitro transcriptional analysis (41). This suggests that different tran-

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scriptional activation domains of the HNF1 polypeptide mediate the increased rate of transcription from HNF1 binding site-containing promoters under different conditions.

MATERIALS AND METHODS

Plasmid constructions. The various steps in the cloning of the plasmid constructs used in the transfection experiments were performed by standard techniques (48). Plasmid SpLUC contains one complete HBV genome (nucleotides 130 to 3182/1 to 133′) located directly 5′ to the promoterless firefly luciferase (LUC) reporter gene so that expression of the LUC gene is governed by the HBV major surface antigen promoter. The designation 133′ is used to indicate that nucleotides 130 to 133 are present twice in this plasmid and that nucleotides 130 to 133 and 130′ to 133′ are distal and proximal to the LUC open reading frame (ORF), respectively. The nucleotide sequences are designated by using coordinates derived from the GenBank genetic sequence data bank. Similarly, the plasmids XpLUC, CpLUC, and PS(1)pLUC, containing one complete HBV genome (nucleotides 1375 to 3182/1 to 1376′, 1805 to 3182/1 to 1804, and 2840 to 3182/1 to 2843′, respectively), were constructed so that expression of the LUC gene is governed by the HBV X gene, nucleocapsid, and large surface antigen promoters, respectively. Details of the construction of these plasmids and the large surface antigen promoter deletion constructs PS(1)pΔ2840-2718LUC, PS(1)pΔ2840-2733LUC, and PS(1)pΔ2840-2767LUC have been described previously (44).

The PS(1)pΔ2840-2767HNF1(+)LUC and PS(1)pΔ2840-2767HNF1(−)LUC plasmids were constructed by cloning a double-stranded oligonucleotide, produced by annealing the oligonucleotides AGCTAGTTAATCATTACTTC and AGC TGAAGTAATGATTAACT, into the unique *Hin*dIII site of plasmid PS(1)pΔ2840-2767LUC (44) in the same orientation (+) or opposite orientation (−) to the HNF1 binding site in the HBV genome. Similarly, the PS(1)pΔ2840-2767AHNF1 (+)LUC plasmid was constructed by cloning a double-stranded oligonucleotide, produced by annealing the oligonucleotides AGCTAGTTAATAATCTACAA and AGCTTT GTAGATTATTAACT, into the unique *Hin*dIII site of the plasmid PS(1)pΔ2840-2767LUC (44) in the same orientation (+) as the HNF1 binding site in the HBV genome.

The pMTHNF1 vector expresses the HNF1 polypeptide from the HNF1 cDNA. pMTHNF1 was constructed by cloning the HNF1 cDNA (21) into the mouse metallothionein-I vector pMT, comprising the promoter sequences from −700 to +64 and the polyadenylation recognition sequence from +930 to +1241 (37) cloned into pUC13. The HNF1 cDNA deletion constructs were produced from the full-length cDNA by using convenient restriction enzyme sites or *Bal*31 nuclease digestion. A termination codon was introduced into the carboxyl-terminal deletions by insertion of an *Nhe*I linker (CTAGCTAGCTAG). The designations of the truncated HNF1 polypeptides indicate which amino acid residues have been deleted from the 628-amino-acid HNF1 polypeptide. The truncated HNF1 polypeptides were expressed by using the mouse metallothionein-I vector pMT as described for the full-length HNF1 polypeptide.

Cells and transfections. The human hepatoma cell lines HepG2, HepG2.1, and Huh7 were grown in RPMI 1640 medium and 10% fetal calf serum at 37°C in 5% CO_2–air and transfected as described previously (37). The transfected DNA mixture comprised 15 μg of a LUC plasmid, 1.5 μg of a pMTHNF1 expression vector or 1.5 μg of the control expression vector pMT, and 1.5 μg of pSV2CAT (25), except where indicated. pSV2CAT directs the expression of the chloramphenicol acetyltransferase gene from the simian virus 40 early promoter and served as an internal control for transfection efficiency. The activity of the simian virus 40 early promoter is not influenced by the presence of exogenously expressed HNF1 polypeptide (43). Cell extracts were prepared 40 to 48 h after transfection and assayed for luciferase and chloramphenicol acetyltransferase activity as reported previously (17, 44, 45).

TABLE 1. Relative activity of HBV promoters in HepG2.1 and Huh7 cells with and without exogenously expressed HNF1 polypeptide[a]

Promoter	Relative activity					
	HepG2.1 cells			Huh7 cells		
	−HNF1	+HNF1	Fold induction	−HNF1	+HNF1	Fold induction
SpLUC	1.00	0.59	0.6	1.00	0.79	0.8
XpLUC	0.83	0.62	0.8	4.49	3.25	0.7
CpLUC	0.18	0.15	0.8	3.91	3.59	0.9
PS(1)pLUC	0.003	0.03	10.0	0.04	0.27	6.8

[a] The expression vectors used were pMTHNF1 (+HNF1) and pMT (−HNF1). The activities of the HBV promoters are reported relative to the activity of the major surface antigen promoter (Sp) in the absence of exogenously expressed HNF1 in each cell line. Xp, X gene promoter; Cp, core antigen promoter; PS(1)p, large surface antigen promoter.

Nuclear extracts and gel retardation analysis. HepG2.1 cells were transfected with 30 μg of the expression vectors encoding various truncated HNF1 polypeptides. Nuclear extracts were prepared from Huh7 cells and HepG2.1 cells as described previously (45). Then, 0.1 ng of ^{32}P-labeled double-stranded large surface antigen promoter HNF1 binding site oligonucleotide (see above under "Plasmid constructions") was incubated with 6 μg of nuclear extract prior to 4% polyacrylamide gel electrophoresis and autoradiography (2).

RESULTS

Influence of HNF1 on transcription from the four HBV promoters. Previously, we have constructed luciferase reporter gene constructs to determine the relative transcriptional activities of the four HBV promoters. The SpLUC, XpLUC, CpLUC, and PS(1)pLUC constructs contain the complete HBV genome located immediately 5′ to the luciferase reporter gene, so that expression of the luciferase reporter gene is controlled by the major surface antigen, X gene, nucleocapsid (core), and large surface antigen (pre-S1) promoters, respectively (44). Synthesis of the HNF1 polypeptide in transient-transfection assays was achieved by using the pMTHNF1 expression vector. pMTHNF1 was constructed by cloning the HNF1 cDNA (21) into the mouse metallothionein-I vector pMT, comprising the promoter sequences from −700 to +64 and the polyadenylation recognition sequence from +930 to +1241 (37).

The effect of expressing the HNF1 polypeptide in the dedifferentiated human hepatoma cell line HepG2.1 and the differentiated human hepatoma cell line Huh7 on the transcriptional activities of the four HBV promoters was determined in transient-transfection assays (Table 1). It is apparent that HNF1 increased transcription from the large surface antigen promoter approximately 10-fold in HepG2.1 cells and 7-fold in Huh7 cells, whereas HNF1 did not influence the transcriptional activities of the other three HBV promot-

ers despite the fact that the SpLUC, XpLUC, and CpLUC constructs possess the HNF1 binding site. This suggests that the binding of the HNF1 polypeptide to the large surface antigen promoter increases transcription from this promoter without affecting transcription from the other HBV promoters, either directly or by transcriptional interference. Increasing the amount of the pMTHNF1 expression vector in these transfection assays increased the transcriptional activation from the large surface antigen promoter (see Fig. 4) without affecting the level of transcription from the other HBV promoters (43).

HNF1 transactivates expression from the HBV large surface antigen promoter through the HNF1 binding site. To establish that expression of the cloned HNF1 cDNA influenced transcription from the large surface antigen promoter through the HNF1 binding site located between −89 and −77 relative to the transcription initiation site of the large surface antigen gene (Fig. 1A), promoter deletion constructs (44) were tested to determine whether they were transactivatable by HNF1 (Fig. 1). In HepG2.1 cells, transcription from the large surface antigen promoter in the constructs PS(1)pLUC and PS(1)pΔ2840-2718LUC (promoter sequence −90 to +35) was transactivated 12- to 15-fold by the HNF1 polypeptide, whereas no transcriptional activation was observed when the PS(1)pΔ2840-2733LUC (promoter sequence −75 to +35) construct, lacking the HNF1 binding site, was examined (Fig. 1B). Similar results were obtained with the differentiated human hepatoma cell lines HepG2 and Huh7, except that the magnitude of the transcriptional induction was reduced, particularly in the HepG2 cell line, presumably reflecting the presence of functional endogenous HNF1 polypeptide in these cells (44) (Fig. 1C). This analysis demonstrated that the HNF1 polypeptide encoded by the HNF1 cDNA activated transcription from the large surface antigen promoter through the HNF1 binding site located between −90 and −76.

Transactivation of transcription by the HNF1 polypeptide mediated through synthetic HNF1 binding sites. As the HNF1 polypeptide has been shown to bind its recognition sequence as a dimer (11, 41), it was of interest to determine whether the orientation of the HNF1 binding site in the large surface antigen promoter influenced its transcriptional activity. This was examined by cloning a single synthetic double-stranded oligonucleotide containing the large surface antigen promoter HNF1 binding site upstream of the HBV sequences in the construct PS(1)pΔ2840-2767LUC (promoter sequence −41 to +35) (Fig. 2). The construct PS(1)pΔ2840-2767HNF1(+)LUC contains the synthetic HNF1 binding site in the same orientation as is found in the HBV genome, and it is located 22 nucleotides from the large surface antigen promoter TATA box sequence (promoter sequence −31 to −25). The construct PS(1)pΔ2840-2767HNF1(−)LUC contains the synthetic HNF1 binding site in the opposite orientation from that found in the HBV genome, and it is located 21 nucleotides from the large surface antigen promoter TATA box sequence. In the HBV genome, and therefore in the constructs PS(1)pLUC and PS(1)pΔ2840-2718LUC, the HNF1 binding site and the TATA box sequence are separated by 45 nucleotides.

The activities of the synthetic large surface antigen promoters in the constructs PS(1)pΔ2840-2767HNF1(+)LUC and PS(1)pΔ2840-2767HNF1(−)LUC in HepG2.1 cells were 16- to 20-fold higher than with the native promoter in the construct PS(1)pΔ2840-2718LUC (Fig. 2). This suggests that the closer proximity of the HNF1 binding site to the TATA box sequence results in higher transcriptional activity from the large surface antigen promoter owing to the more efficient utilization of the residual endogenous HNF1 polypeptide or HNF1-related polypeptides present in the HepG2.1 cells (Fig. 3A, lane 3). Consistent with the suggestion that HNF1 binds its recognition sequence as a homodimer, the transcriptional activities from the two synthetic large surface

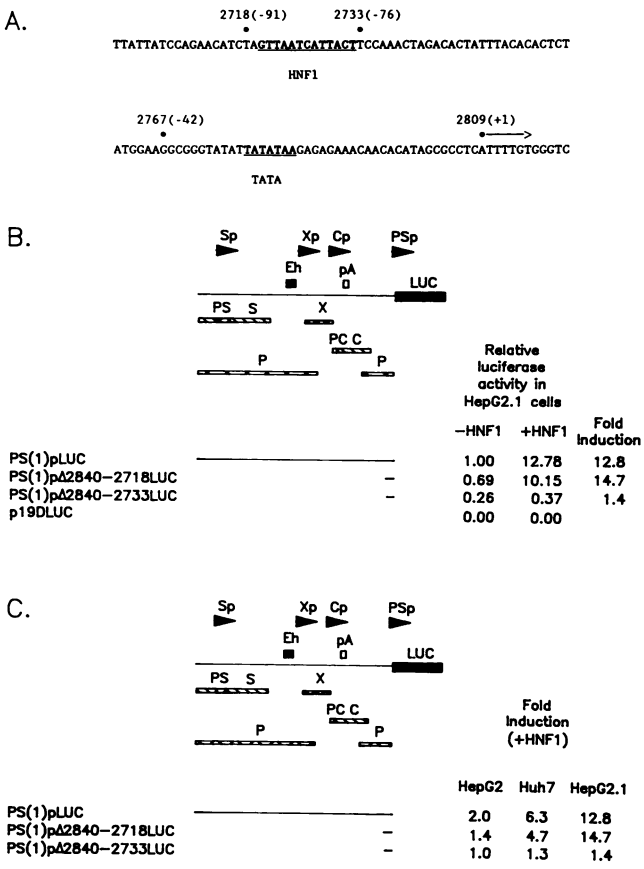

FIG. 1. (A) Sequence of the HBV large surface antigen promoter region (subtype *ayw*) (22). The numbered nucleotides (coordinates derived from the GenBank genetic sequence data bank) indicate the breakpoints of the functionally significant deletions. The numbers in parentheses indicate the locations of the breakpoints relative to the HBV large surface antigen transcription initiation site (49, 60), which is designated by an arrow. The underlined sequences represent the HNF1 binding site and TATA box sequence homologies. (B) Influence of the HNF1 polypeptide in HepG2.1 cells on expression from various large surface antigen promoter constructs, which included [PS(1)pLUC and PS(1)pΔ2840-2718LUC] or were deleted for [PS(1)pΔ2840-2733LUC] the HNF1 binding site. The relative activities of the large surface antigen promoter constructs were determined in the presence (+HNF1) and the absence (−HNF1) of exogenously expressed HNF1 polypeptide with the expression vectors pMTHNF1 and pMT, respectively. Arrows indicate the positions and direction of transcription from the HBV surface (Sp), X gene (Xp), core (Cp), and pre-S1 (PSp) promoters. Boxes indicate the positions of the HBV enhancer sequence (Eh), HBV polyadenylation sequence (pA), pre-surface antigen ORF (PS), surface antigen ORF (S), X gene ORF (X), precore ORF (PC), core ORF (C), polymerase ORF (P), and luciferase ORF (LUC). The horizontal lines indicate the HBV sequences present in the various PS(1)p plasmids. The HBV sequences deleted from the various plasmids are designated by nucleotide coordinates. (C) Influence of expression of the HNF1 polypeptide on the transcriptional activity of the large surface antigen promoter in HepG2, Huh7, and HepG2.1 cells.

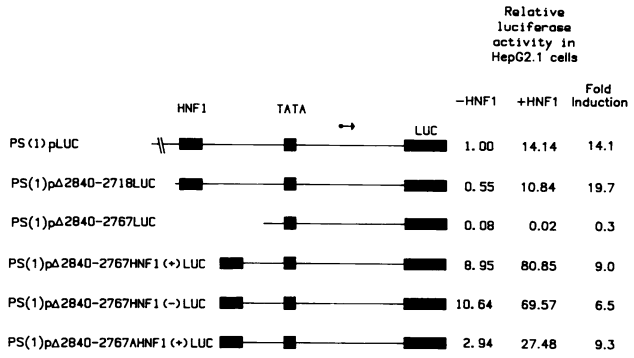

FIG. 2. Influence of the orientation and sequence of the HNF1 binding site on transcriptional activity from large surface antigen minimal promoter constructs containing single synthetic HNF1 binding sites in HepG2.1 cells. The relative activities of the minimal promoter constructs were determined in the presence (+HNF1) and the absence (−HNF1) of exogenously expressed HNF1 polypeptide with the expression vectors pMTHNF1 and pMT, respectively.

antigen promoters with the HNF1 binding site in either orientation were approximately equivalent. In addition, expression of exogenous HNF1 polypeptide stimulated transcription 6- to 20-fold from each of the large surface antigen promoter constructs containing the HNF1 binding site (Fig. 2). This result demonstrated that the HNF1 polypeptide encoded by the cloned HNF1 cDNA stimulated transcription through a synthetic HNF1 binding site and that its ability to stimulate transcription was not restricted by the distance between the HNF1 binding site and the TATA box sequence.

It was also of interest to determine whether the cloned HNF1 cDNA encoded a polypeptide capable of transactivating transcription from HNF1 binding sites of different sequence. This was examined by cloning the human albumin promoter HNF1 binding site into the PS(1)pΔ2840-2767LUC minimal promoter construct as a synthetic oligonucleotide in a manner similar to that used to generate the PS(1)pΔ2840-2767HNF1(+)LUC construct. This generated the construct PS(1)pΔ2840-2767AHNF1(+)LUC, which has the human albumin promoter HNF1 binding site located 22 nucleotides from the large surface antigen promoter TATA box sequence. The human albumin promoter HNF1 binding site (GTTAATAATCTAC) differs from the HBV large surface antigen binding site (GTTAATCATTACT) at 4 of the 12 conserved positions in the consensus HNF1 binding site (GTTAATNATTAAC) (13). However, both promoter binding sites differ from the consensus HNF1 binding site at only two adjacent nucleotides located in the 3′ half of the sequence. As observed for the PS(1)pΔ2840-2767HNF1(+) LUC construct, the PS(1)pΔ2840-2767AHNF1(+)LUC construct displayed transcriptional activity which was induced approximately 10-fold by the expression of exogenous HNF1 polypeptide (Fig. 2). This demonstrated that the human albumin promoter HNF1 binding site could mediate the transcriptional activation induced by the expression of the cloned HNF1 cDNA in HepG2.1 cells in a manner quantitatively similar to that observed for the large surface antigen HNF1 binding site in the context of a minimal promoter construct.

Characterization of the HNF1 polypeptide transcriptional activation domain. The functional domains of the HNF1 polypeptide have been examined, and the dimerization domain has been located in the 31 amino-terminal amino acids, the DNA-binding domain (POU-A and homeoboxlike domains) has been located in the 281 amino-terminal amino acids, and the in vitro transcription transactivation domains have been located between amino acid residues 281 and 318 and residues 547 and 628 (11, 21, 41). The transcriptional activation domain of the HNF1 polypeptide responsible for the stimulation of transcription from the large surface antigen promoter [PS(1)pLUC] and the minimal promoter constructs containing the large surface antigen or human albumin promoter HNF1 binding sites [PS(1)pΔ2840-2767HNF1(+)LUC and PS(1)pΔ2840-2767AHNF1(+)LUC, respectively] were determined by transient transfections in HepG2.1 cells (Fig. 3). Since the dimerization and DNA-binding

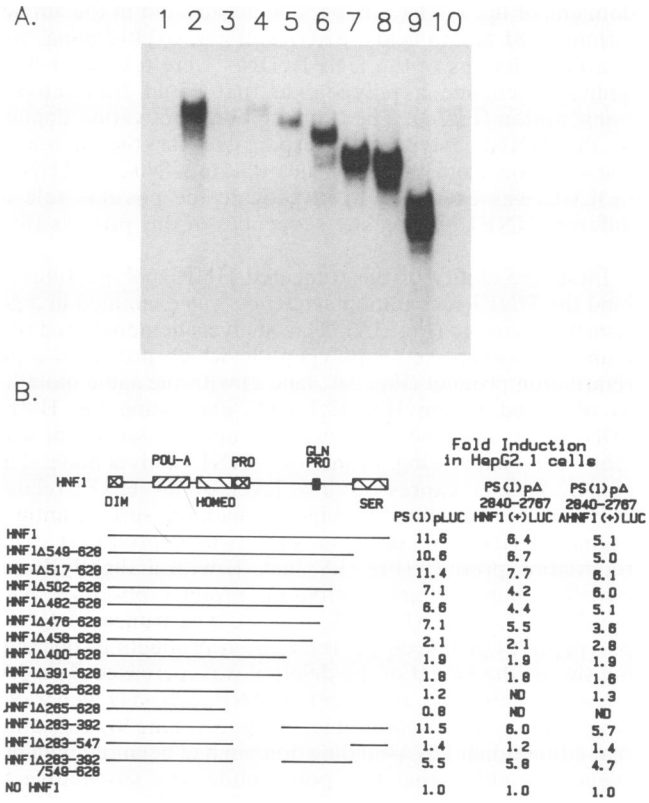

FIG. 3. (A) Gel retardation analysis of truncated HNF1 polypeptides. HepG2.1 cells were transfected with 30 μg of the expression vector (lane 4) pMTHNF1, (lane 5) pMTHNF1Δ502-628, (lane 6) pMTHNF1Δ458-628, (lane 7) pMTHNF1Δ400-628, (lane 8) pMTHNF1Δ391-628, (lane 9) pMTHNF1Δ283-547, or (lane 10) pMT. Nuclear extracts were prepared from (lane 2) Huh7 cells and (lanes 3 to 10) HepG2.1 cells. Lane 1 is a control in which the HNF1 oligonucleotide was incubated with buffer instead of nuclear extract. (B) Deletion analysis of the HNF1 polypeptide and identification of the domain involved in transcriptional activation of the large surface antigen promoter [PS(1)pLUC] and minimal promoter constructs containing synthetic HNF1 binding sites [PS(1)pΔ2840-2767HNF1(+)LUC and PS(1)pΔ2840-2767AHNF1(+)LUC] in HepG2.1 cells. The large surface antigen promoter reporter plasmid used was PS(1)pLUC. DIM, dimerization domain (11, 41); POU-A, POU-A domain homology (4, 11, 21); HOMEO, homeoboxlike domain (4, 11, 21); PRO, proline-rich transcriptional activation domain (41); GLN/PRO, glutamine- and proline-rich transcriptional activation domain; SER, serine-rich transcriptional activation domain (41). ND, not done.

domains of the HNF1 polypeptide are located in the amino-terminal 281 amino acids, a series of carboxyl-terminal and internal deletions of the HNF1 cDNA were tested for their ability to encode a polypeptide that could transactivate transcription (Fig. 3). The transcriptional activation domain of the HNF1 polypeptide responsible for the increased transcription from the construct PS(1)pΔ2840-2767AHNF1 (+)LUC was examined to investigate the possible role of different HNF1 binding-site sequences in this process (Fig. 3).

First, the ability of the truncated HNF1 polypeptides to bind the HNF1 recognition sequence was examined in a gel retardation assay (Fig. 3A). This analysis demonstrated that Huh7 cells expressed a polypeptide which produced a gel retardation product (Fig. 3A, lane 2) with the same mobility as observed in the HepG2.1 cells expressing the HNF1 cDNA (Fig. 3A, lane 4). This observation is consistent with Huh7 cells expressing a functional HNF1 polypeptide. The HepG2.1 cells express a low level of an HNF1-related polypeptide (as defined by binding the large surface antigen promoter HNF1 recognition site), which produced a gel retardation product (Fig. 3A, lane 3) with a slightly faster mobility than the gel retardation product observed with Huh7 cell extracts (Fig. 3A, lane 2). The truncated HNF1 polypeptides produced gel retardation products of decreasing size as the extent of the deletion was increased (Fig. 3A, lanes 5 to 9). The polypeptide HNF1Δ283-547 generated several gel retardation products (Fig. 3A, lane 9), indicating that a functional DNA-binding domain had been retained but suggesting either that this polypeptide was susceptible to proteolytic degradation or that it may fold into multiple distinct conformations.

The functional analysis of the truncated HNF1 polypeptides indicated that the carboxyl-terminal 112 amino acids of HNF1 (HNF1Δ517-628) were nonessential for maximal levels of transactivation of each of the promoter constructs examined (Fig. 3B). Amino acids between residues 502 (HNF1Δ502-628) and 516 (HNF1Δ517-628) appear to be necessary to achieve maximal levels of transcriptional activation from the large surface antigen promoter HNF1 binding site, as deletion of these residues resulted in approximately a 35% reduction in transactivation. Deletion of the next 26 carboxyl-terminal amino acids (HNF1Δ476-628 and HNF1Δ482-628) did not influence the ability of the truncated HNF1 polypeptides to transactivate the large surface antigen promoter. However, the additional carboxyl-terminal deletion of amino acid residues 458 to 475 from the HNF1 polypeptide (HNF1Δ458-628) resulted in loss of the majority of the transactivational activity of the truncated HNF1 polypeptide (Fig. 3B). This 18-amino-acid region of the HNF1 polypeptide contains five glutamine residues and four proline residues. Although the significance of this observation is unclear, it is consistent with previous reports that the transcriptional activation domains of the transcription factors Sp1 and CTF are rich in glutamine and proline residues, respectively (12, 39). This analysis identified a major transcriptional activation domain of HNF1 between amino acids 458 and 475, which is distinct from the previously identified transcriptional activation domains of the HNF1 polypeptide (amino acid residues 281 to 318 and 547 to 628) determined by in vitro transcription analysis with a promoter containing human albumin HNF1 binding sites (41). However, analysis of the transactivation domain responsible for the increase in transcription from the minimal promoter construct containing the human albumin promoter HNF1 binding site [PS(1)pΔ2840-2767AHNF1(+)LUC] in transient-transfection assays revealed that amino acid residues from 400 (HNF1Δ400-628) to 481 (HNF1Δ482-628) mediated this effect (Fig. 3B). This region encompasses the principal transcriptional activation domain mapped by using the large surface antigen promoter constructs but is distinct from the previously reported transcriptional activation domains of the HNF1 polypeptide (41).

In an attempt to confirm the observed difference in the mapping of the transactivation domains of HNF1 in these two systems, three internally deleted HNF1 polypeptides were examined for their ability to transactivate transcription from the various promoter constructs (Fig. 3B). Deletion of amino acid residues 283 to 392 (HNF1Δ283-392) did not influence the ability of the HNF1 polypeptide to transactivate these promoters, whereas deletion of amino acid residues 283 to 547 (HNF1Δ283-547) essentially eliminated the transactivation function of the HNF1 polypeptide. These results and the observation that the carboxyl-terminal truncation of 112 amino acids (HNF1Δ517-628) does not affect the transactivation activity of HNF1 are consistent with the essential nature of amino acid residues 393 to 516 and the nonessential role of the previously identified transcriptional activation domains in the transactivation of these promoters in this system. However, to establish definitely that the previously identified transcriptional activation domains (41) were not required for the transactivation of these promoters in this system, an HNF1 polypeptide (HNF1Δ283-392/549-628) lacking both of these regions was shown to transactivate these promoters by approximately five- to sixfold (Fig. 3B). This demonstrated that amino acid residues between 393 and 548 were sufficient to transactivate the large surface antigen promoter through either the large surface antigen or the human albumin HNF1 binding site when they were joined to the DNA-binding domain (residues 1 to 282) of the HNF1 polypeptide. This result indicates that the transcriptional activation domain responsible for the induction of transcription from HNF1 binding site-containing promoters in these transfection experiments is distinct from the transcriptional activation domain mapped by using the human albumin HNF1 recognition sequence in in vitro transcription analysis (41).

As the HNF1 expression vectors might result in the synthesis of different amounts of the various truncated HNF1 polypeptides, the effect of varying the amount of the HNF1 expression vectors on the transcriptional activation of the large surface antigen promoter in HepG2.1 cells was examined (Fig. 4). This analysis showed that the pMTHNF1, pMTHNF1Δ549-628, and pMTHNF1Δ283-392/549-628 expression vectors displayed similar dose-dependent transcriptional activation of the large surface antigen promoter. The pMTHNF1Δ400-628, pMTHNF1Δ283-547, and pMTHNF1Δ283-628 expression vectors produced minimal effects on the transcriptional activity of the large surface antigen promoter. This demonstrates that the amount of the expression vector transfected in these assays does not influence the mapping of the transcriptional activation domain of the HNF1 polypeptide. The reduced level of transcriptional activation observed in this analysis at low expression vector levels (1.5 μg) can, in part, be explained by the higher amount of DNA used in these transfection assays (46.5 μg) than in the standard transfection assays (16.5 μg).

DISCUSSION

Analysis of the HBV large surface antigen promoter indicated that a binding site for the HNF1 polypeptide

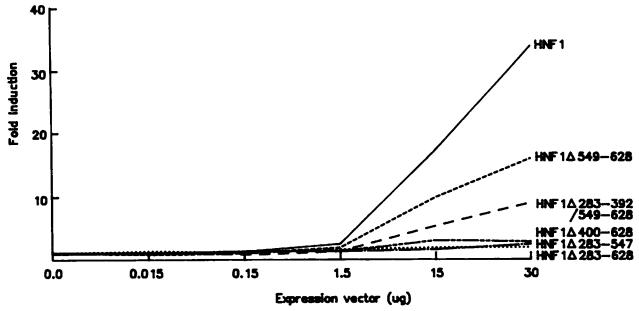

FIG. 4. Effect of expression vector levels on transcriptional activation of the large surface antigen promoter in HepG2.1 cells. Cells were transfected with 15 μg of PS(1)pLUC, 1.5 μg of pSV2CAT, and 30 μg of pMT plus expression vector DNA. The indicated amount of expression vector was combined with pMT DNA so that the total amount of DNA was 30 μg. Each transfection mixture therefore contained a total of 46.5 μg of DNA.

represented an important regulatory sequence element of this promoter (10, 13, 40, 44). Therefore, the cloning of the cDNA encoding the HNF1 polypeptide (4, 11, 21) permitted detailed examination of the role of this transcription factor in the regulation of the large surface antigen promoter and the other three HBV promoters. By using transient-transfection assays, it was shown that the HNF1 polypeptide expressed from the cloned HNF1 cDNA increased transcription from the large surface antigen promoter but did not influence transcription from the other HBV promoters (Table 1). This demonstrated that HNF1 can regulate the expression of the large surface antigen transcript independently of the other HBV transcripts. Since the large envelope polypeptide is an essential component of the HBV envelope (27), modulation of the level of expression of this polypeptide by HNF1 could influence the biogenesis of the virion.

The HNF1 binding site located between −89 and −77 relative to the initiation site for transcription of the large surface antigen RNA was shown to mediate the increased level of transcription from the large surface antigen promoter resulting from the expression of the cloned HNF1 cDNA (Fig. 1). In addition, synthetic oligonucleotides containing HNF1 binding sites were shown to mediate the HNF1-induced increase in the level of transcription from a minimal promoter construct (Fig. 2). The spacing between the HNF1 binding site and the TATA box binding site is 45 nucleotides in the case of the large surface antigen promoter and 21 or 22 nucleotides in the case of the minimal promoter constructs. However, the increase in the level of transcription induced by HNF1 was similar in each case, so that the distance between these binding sites appears to be unimportant in determining the level of induction, although it does affect the uninduced level of transcription. This analysis also indicated that the large surface antigen promoter and human albumin promoter HNF1 binding sites were equally efficient at mediating the induction of transcription by the HNF1 polypeptide from a minimal promoter construct in HepG2.1 cells. In addition, since the large surface antigen minimal promoter constructs lack an Oct-1 binding site, it is apparent that, in this system, the HNF1 polypeptide can induce transcription independently of the Oct-1 binding site. This differs from the previously reported requirement for Oct-1 for induction of the large surface antigen promoter by the HNF1 polypeptide (62). The reasons for this difference are unclear but may reflect differences in the systems used to analyze the large surface antigen promoter.

The ability of the HNF1 polypeptide to transactivate transcription from HNF1 binding site-containing promoters permitted identification of the protein domain involved in mediating this process (Fig. 3). The transcriptional activation domain of the HNF1 polypeptide was located between amino acid residues 393 and 548. The 18 amino acids between residues 458 and 475 appeared to represent a critical region involved in transactivation of the promoters containing the HNF1 binding site present in the large surface antigen promoter. This region is rich in glutamine and proline residues (50%), which is similar to the observation that the transcriptional activation domains of the transcription factors Sp1 and CTF are rich in glutamine and proline residues, respectively (12, 39). The transcriptional activation domain of the HNF1 polypeptide mapped between amino acid residues 400 and 481 when a minimal promoter construct containing the human albumin promoter HNF1 binding site was used for this analysis. This region is larger than, but includes, the critical region identified with the large surface antigen promoter HNF1 binding-site constructs. This region is also rich in glutamine and proline residues (20 of 82 amino acids), suggesting the possible importance of these amino acids in mediating the transcriptional activation process.

The transcriptional activation domain identified in this study by using transient-transfection assays is located within amino acid residues 393 to 548. However, the transcriptional activation domains of HNF1 have previously been mapped in an in vitro transcription system between residues 281 and 318 and residues 547 and 628 (41). Therefore, these studies have mapped the transcriptional activation domains of the HNF1 polypeptide to mutually exclusive regions of this transcription factor. This is an interesting observation because the mapping of the transcriptional activation domains of two transcription factors, Sp1 (12, 32) and c-Jun (3), in both transient-transfection assays and in vitro transcription systems has identified the same polypeptide domains as mediating transcriptional activation.

There are several possible explanations for the observed difference in the mapping of the transcriptional activation domain of the HNF1 polypeptide. Since both studies determined the transactivation domain of HNF1 bound to the human albumin HNF1 binding site, differences in the sequence of the HNF1 binding sites cannot account for the observed differences in the identified transcriptional activation domains of the protein in these systems. In addition, since the distance between the HNF1 binding site and the TATA box binding site did not influence the region of the HNF1 polypeptide responsible for transcriptional activation, it appears unlikely that the spatial relationship between HNF1 and the general transcription initiation machinery is responsible for these observations. Therefore, it seems probable that the differences between the transfection analysis used in this study and the previously reported in vitro transcription analysis (41), such as the cell types and assay systems employed, must account for the observed differences in the mapping of the transcriptional activation domains. It is possible that cell type-specific coactivator molecules could explain these observations if different coactivators recognize distinct regions of the HNF1 polypeptide and subsequently increase the transcriptional activity of the general transcription machinery.

Recently, the cDNA for the transcription factor vHNF1, which is closely related to HNF1, has been cloned and

characterized (16, 38, 46). vHNF1 can form homodimers and heterodimers with HNF1, binds to HNF1 recognition sequences, and displays transcriptional activation properties very similar to those of HNF1 (16, 46). Interestingly, the previously described transcriptional activation domains of HNF1 (41) are not conserved between HNF1 and vHNF1 (16, 38, 46). However, the region of the HNF1 polypeptide containing the transcriptional activation domain identified in this study and located between residues 393 and 548 displays 54% amino acid identity (71 of 132 residues) with the vHNF1 polypeptide (16). In addition, the 18 amino acids located between residues 458 and 475, which are critical for transcriptional activation of the large surface antigen promoter, have 78% amino acid identity between HNF1 and vHNF1. These observations suggest that these conserved regions of the HNF1 and vHNF1 polypeptides might be responsible for the similar transcriptional transactivation properties of these transcription factors in transient-transfection assays (16, 46).

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