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Regulation of Hepatitis B Virus Core Promoter by Transcription Factors HNF1 and HNF4 and the Viral X Protein

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Hepatitis B virus (HBV) core promoter contains a binding site for nuclear receptors. A natural double mutation in this binding site, which changes nucleotide (nt) 1765 from A to T and nt 1767 from G to A, selectively abolishes the binding of several nuclear receptors without affecting that of HNF4. This double mutation also creates a binding site for the transcription factor HNF1 and changes two amino acids in the overlapping X protein sequence. In this study, we have examined the roles of HNF1, HNF4, and the X protein in the regulation of the core promoter activities in Huh7 hepatoma cells. Our results indicate that HNF4 could stimulate the expression of the precore RNA and the core RNA from the core promoter of both the wild-type (WT) HBV and the double mutant, although its effect on the former was more prominent. In contrast, HNF1, which did not affect the WT core promoter, suppressed the precore RNA expression of the double mutant. Further analysis using HBV genomic constructs, with and without the ability to express the X protein, indicates that the X protein did not affect the HNF4 activity on the core promoter and affected the HNF1 activity on the core promoter of only the double mutant. Thus, our results indicate that the phenotypic differences of HBV WT and double-mutant core promoters are at least partially due to the differential activities of HNF1, HNF4, and the X protein on these two promoters.

Hepatitis B virus (HBV) is a small enveloped virus that belongs to the hepadnavirus family (4). This virus infects approximately 350 million people in the world. The genome of HBV is a 3.2-Kb circular and partially double-stranded DNA molecule. Upon infection, the viral genome is delivered to the nucleus, where it is repaired to become a covalently closed circular DNA molecule. This covalently closed circular DNA then serves as the template for the transcription of viral RNAs. The transcription of HBV RNAs is controlled by four different promoters. The pre-S1 promoter and the major S promoter control the transcription of viral RNAs that encode the three related envelope proteins known as pre-S1, pre-S2, and major S proteins. The X promoter controls the transcription of the X protein RNA. The X protein is a regulatory protein that has pleiotropic functions. The core promoter directs the transcription of two viral RNAs termed the precore RNA and the core RNA. The precore RNA codes for the precore protein, which is the precursor of the circulating e antigen found in HBV-infected patients. This precore protein is thought to be important for the establishment of chronic infection and has been shown to suppress HBV replication (6, 15). The core RNA codes for the core protein and the viral DNA polymerase, which is also a reverse transcriptase. The core protein is a structural protein. It packages its own mRNA, which is also known as the pregenomic RNA, to form the core particle. The increase of the core RNA expression has been shown to increase the viral replication rate (7, 9).

In addition to the four promoters, the transcription of the HBV genome is also regulated by two enhancer elements known as EN1 and EN2 (21). A number of transcription factors, including nuclear receptors, regulate the activities of HBV promoters and enhancers. There are three known nuclear receptor binding sites in the viral genome: one in the EN1 enhancer (5, 10), one in the EN2 enhancer (8), and one in the core promoter (3, 16, 23). The nuclear receptor binding site in the core promoter is recognized by COUP-TF1, COUP-TF2, the PPARα-RXRα heterodimer, testicular receptor 2 (TR2), testicular receptor 4 (TR4), and the liver-enriched transcription factor HNF4 (3, 14, 16, 22). These nuclear receptors exhibit different effects on the core promoter activities. For example, COUP-TF1, COUP-TF2, TR2, and TR4 can suppress the expression of the precore RNA or both the precore RNA and the core RNA (14, 24), and PPARα-RXRα and HNF4 can enhance the expression of both precore and core RNAs (16, 17, 24). As the precore RNA and the core RNA possess very different biological functions, differential regulation of the core promoter activities by these nuclear receptors may have profound effects on HBV replication and pathogenesis.

A natural double mutation is frequently detected in the nuclear receptor binding site of the core promoter. This double mutation, which invariably changes nucleotide (nt) 1765 from A to T and nt 1767 from G to A (Fig. 1), suppresses the precore RNA transcription and modestly increases the viral replication rate (2, 3, 7, 17, 19). This double mutant is frequently isolated from HBV patients with chronic hepatitis symptoms (1). Recent studies indicate that this double mutation abolishes the binding of COUP-TFs, PPARα-RXRα, and TR4 to the core promoter without affecting the binding by HNF4 (3, 19, 22). Interestingly, this double mutation also creates a binding site for the liver-enriched transcription factor

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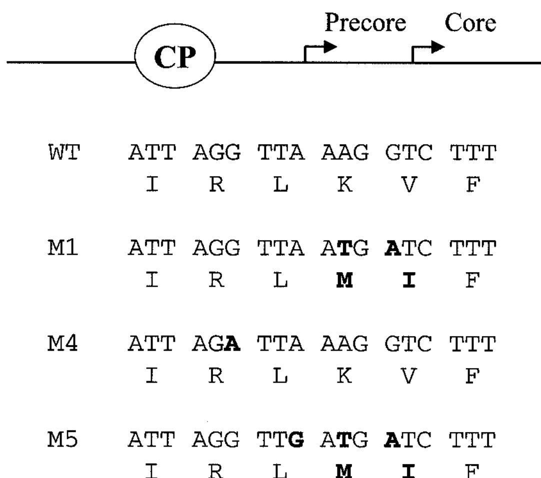

FIG. 1. HBV core promoter sequences of various mutants. The core promoter (CP) and the transcription initiation sites of precore and core RNAs are illustrated. Part of the core promoter sequences of WT, M1, M4, and M5 are compared. The amino acid sequence of the X protein is also shown. The boldface letters highlight the locations of nucleotide and amino acid mutations.

HNF1 (11, 19). Since the core promoter overlaps with the X protein coding sequence in the HBV genome, this double mutation also changes two codons in the X protein coding sequence (Fig. 1). Our recent studies indicate that the mutated X protein can physically and functionally interact with HNF1 to regulate the core promoter activity (13).

In this report, we have further investigated the possible roles of HNF1, HNF4, and the X protein in the regulation of the core promoter activities. Our results indicate that HNF4 can stimulate the transcription of the precore RNA and the core RNA from the wild-type (WT) core promoter and the double-mutant core promoter. In contrast, HNF1, which has no effect on the WT core promoter, suppresses the precore RNA transcription and modestly increases the core RNA transcription from the double-mutant core promoter. Further analysis using HBV mutants with and without the ability to express the X protein reveals distinct effects of HNF1, HNF4, and the HBV X protein on the core promoter activities.

MATERIALS AND METHODS

DNA plasmids. The constructions of the HBV genomic constructs pWTD, pM1D, pM4D, and pM5D as well as pCMV-HNF1 have been described before (12). pM1DX$^-$, pM4DX$^-$, and pM5DX$^-$ are identical to pM1D, pM4D, and pM5D, respectively, except that they contain an A-to-C mutation at nt 1377 and a C-to-T mutation at nt 1398. These two mutations abolish the X protein expression without affecting the overlapping polymerase coding sequence. The HNF4 expression plasmids, pCMV-HNF4S (sense clone) and pCMV-HNF4AS (antisense clone), were constructed by inserting the BamHI fragment of pLEN4S (18) in either sense or antisense orientation into the pCDNA3 vector (Invitrogen) under the expression control of the T7 promoter and the immediate early promoter of cytomegalovirus (CMV). This BamHI fragment contains the rat HNF4 cDNA sequence. The plasmid pCMV-HNF1, which expresses HNF1 by use of the CMV promoter, has been described before. pXGH5 (Nichols Diagnostics) is an expression plasmid for the human growth hormone (hGH). The expression of hGH in this plasmid is under the control of the mouse metallothionine promoter. This plasmid serves as a cotransfection control for monitoring the transfection efficiency. The plasmid pXGH5 has no effect on the core promoter activities, since excluding the plasmid from the transfection experiments did not affect the results (25; also data not shown).

Cell line and DNA transfection. Huh7 cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Cells in a 60-mm-diameter petri dish were grown to 80% confluence and transfected with a total of 10 μg of DNA by the calcium phosphate precipitation method. Forty-eight hours after transfection, the incubation medium was harvested and analyzed for hGH by using a radioimmunoassay kit (Nichols Diagnostics). Cells were then lysed for RNA isolation.

Primer extension analysis. Total cellular RNA was isolated with Trizol reagent (Invitrogen) following the manufacturer's protocol. Ten micrograms of total RNA was used for each primer extension analysis. The oligonucleotide primer used for analyzing HBV precore and core RNA transcripts was 5′GGT GAGCAATGCTCAGGAGACTCTAAGG3′. This sequence is an antisense sequence corresponding to nt 2052 to 2025 of the HBV genome. The oligonucleotide primer used for analyzing the hGH RNA transcript was 5′GCCACTGCA GCTAGGTGAGCGTCC3′. The primer, in the amount of 20 pmol, was labeled with 300 μCi of [γ-^{32}P]-ATP (ICN Pharmaceuticals, Inc.) and 2 μl (5 units/μl) of T4 polynucleotide kinase (Promega) in a 20-μl reaction volume at 37°C for 1 h. The ^{32}P-labeled primer was then purified on a 5% nondenaturing polyacrylamide gel. The primer extension analysis was carried out by using previously described procedures (11). The signals on the autoradiographs were analyzed and quantified by SigmaScan Pro5. All the experiments were repeated multiple times, and the results presented here represent the averages of those experiments.

EMSA. Both pCMV-HNF4S and pCMV-HNF4AS were linearized with the restriction enzyme XhoI and used for RNA synthesis in vitro by using the T7 RNA polymerase. Approximately 0.5 μg of RNA thus synthesized was used for in vitro translation in a final volume of 10 μl by using the rabbit reticulate lysates (Promega). These translation mixtures were then used for the electrophoretic mobility shift assay (EMSA). The sequences of the oligonucleotide probes used for EMSA were as follows: M1, 5′ GAGGAGATTAGGTTAATGATCTTTG TATT 3′ and 3′ CTCTAATCCAATTACTAGAAACATAATC 5′; M4, 5′ GA GGAGATTAGATTAAAGGTCTTTGTATT 3′ and 3′ CTCTAATCTAATTT CCAGAAACATAATC 5′; and M5, 5′ GAGGAGATTAGGTTGATGATCTT TGTAT 3′ and 3′ CTCTAATCCAACTACTAGAAACATAATC 5′.

The differences in these nucleotide sequences are highlighted in Fig. 1. These oligonucleotides were labeled with ^{32}P by using T4 kinase and purified as described above. For EMSA, 1 μl of the translation mixture derived from either pCMV-HNF4S or pCMV-HNF4As was mixed with 1 μl of poly(dI-dC) (1 μg/μl), 1 μl of salmon sperm DNA (1 μg/μl), and 4 μl of 5× Stephan's buffer (2) in a final volume of 19 μl and incubated on ice for 10 min. One microliter (approximately 10^4 cpm) of the DNA probe was then added into each reaction mixture, which was further incubated on ice for 20 min. The translation mixture derived from the antisense construct pCMV-HNF4AS served as a negative control in this experiment. The samples were then subjected to electrophoresis at 4°C on a 5% nondenaturing polyacrylamide gel as previously described (2). For the competition assay, the nonlabeled oligonucleotide competitor was added during the incubation on ice prior to the addition of the WT DNA probe. The sequences of the nonspecific competitor used in the assay were 5′-TGTTTAAGGACGGGG AGGAGATGGGGG-3′ and 3′-AATTCCTGCCCCTCCTCTACCCCCTCC-5′.

RESULTS

Effects of HNF4 on the core promoter of WT and mutant HBV. In order to understand how HNF4 may affect the core promoter activities of the WT HBV and the double mutant (M1), we cotransfected the HBV WT or M1 genomic dimer with an increasing concentration of the HNF4 expression plasmid pLEN45 into Huh7 hepatoma cells. Cells were lysed 48 h after transfection for the isolation of total cellular RNA. HBV precore RNA and core RNA levels were then quantified by primer extension analysis. As shown in Fig. 2, HNF4 increased the WT precore RNA level approximately threefold and the core RNA level approximately sevenfold. HNF4 similarly increased the precore RNA and the core RNA levels of the M1 double mutant, although its effect on M1 was more modest, up to nearly twofold for the precore RNA and slightly more than threefold for the core RNA (Fig. 2). The effects of HNF4 on the core promoter were specific, as HNF4 did not affect the hGH RNA level expressed from the plasmid pXGH5 (Fig. 2),

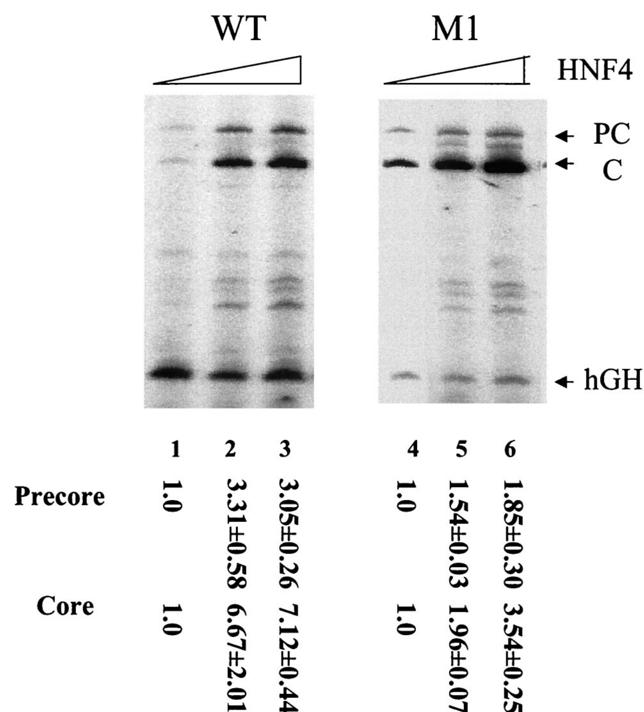

FIG. 2. Effects of HNF4 on the HBV core promoter. Huh7 cells were cotransfected with 4 μg of pWTD (the WT HBV genome) (lanes 1 to 3) or pM1D (the double-mutant genome) (lanes 4 to 6) and various concentrations of the HNF4 expression plasmid pLEN45. Two micrograms of pXGH5 was also included in the cotransfection experiment to monitor the transfection efficiencies (see Materials and Methods for details). Lanes 1 and 4, no pLEN45 was used for the cotransfection; lanes 2 and 5, 2 μg of pLEN45 was used; and lanes 3 and 6, 4 μg of pLEN45 was used. The control vector pLEN0 was used to bring the total amount of DNA to 10 μg for transfection. The locations of the precore RNA, the core RNA, and the hGH RNA are indicated with arrows. The quantification was conducted by use of SigmaScan Pro5. The intensities of the precore and the core RNA bands were normalized against the hGH RNA internal control. The numbers shown represent the precore RNA and the core RNA levels relative to those without the coexpression of HNF4. The experiments were repeated three times, and the results shown represent the averages ± standard errors of those experiments. Note that the intensities of the RNA signals can only be compared within one set of experiments but not between different sets of experiments, due to the differences in the specific activity of the ^{32}P-labeled primers used in different primer extension experiments.

which was used as an internal control in the cotransfection studies. These results indicated that HNF4 could specifically stimulate the core promoter activities of WT and M1.

To understand whether the HNF4 binding site in the core promoter is important for the stimulatory effect of HNF4, we used two core promoter mutants that we had previously created. The M4 mutant is identical to the WT, with the exception of one nucleotide mutation at nt 1760 (Fig. 1). This mutation prevents the binding of COUP-TF (11). The M5 mutant is identical to M1, with the exception of one additional mutation at nt 1763 (Fig. 1). This mutation prevents the binding of HNF1 (11). To test whether HNF4 could bind to these mutated sequences, we performed the electrophoretic mobility assay with oligonucleotide probes. HNF4 was synthesized in vitro by using the rabbit reticulocyte lysates (Fig. 3A). As shown in Fig. 3B, HNF4 was able to bind to M1 and M4 oligonucleotide probes, but its ability to bind to the M5 oligonucleotide probe was greatly diminished. This result was also confirmed in the competition assay. In this assay, the binding of HNF4 to the WT probe was conducted in the presence of different molar ratios of nonlabeled WT, M1, M4, and M5 oligonucleotides. As shown in Fig. 3C, although nonlabeled WT, M1, and M4 oligonucleotides suppressed the binding of HNF4 to the WT probe, the M5 oligonucleotide did not. Furthermore, this result also revealed that the binding affinity of HNF4 to the WT sequence was the highest, followed by that to M4 and then by that to M1.

The HBV genomic dimer containing either the M4 or the M5 sequence was then cotransfected with the HNF4-expression plasmid into Huh7 cells. The expression of precore and core RNAs was then analyzed by primer extension. As shown in Fig. 4, HNF4 increased the precore RNA level in a dose-dependent manner to approximately threefold and the core RNA level to approximately fivefold for the M4 mutant. This result is not surprising, since the M4 core promoter contains the HNF4 binding site. Interestingly, it also increased the precore RNA level of the M5 mutant in a dose-dependent manner to nearly threefold and its core RNA level to more than sixfold. As the M5 core promoter lacks the HNF4 binding site, this result indicated that the HNF4 binding site in the core promoter was not needed for HNF4 to increase the precore RNA and the core RNA levels.

As M5 also differed from WT and M4 in the X protein sequence by two amino acids due to the double mutation (Fig. 1), it remains a possibility that this mutated M5 X protein enabled HNF4 to stimulate the M5 core promoter in the absence of its binding site in this promoter. To rule out this possibility, we tested the effect of HNF4 on M4 and M5 genomic constructs that cannot express the X protein. Two nucleotide mutations were introduced into the HBV genome to abolish the expression of the 16.5-kDa X protein. One mutation removed the ATG codon of the X gene, and the other created a premature termination codon in the X protein coding sequence (20). These two mutations do not affect the overlapping polymerase coding sequence. As shown in Fig. 4, abolishing the expression of the X protein did not significantly affect the ability of HNF4 to stimulate the M4 and M5 core promoters. Thus, the mutations in the X protein sequence did not play a role in enabling HNF4 to stimulate the core promoter in the absence of the HNF4 binding site.

Effects of HNF1 on the core promoter of WT and mutant HBV. As the M1 core promoter also contains an HNF1 binding site, we examined the possible role of HNF1 in the regulation of the M1 core promoter activities. To this end, the M1 genome was cotransfected with an HNF1 expression plasmid into Huh7 cells. The precore RNA and the core RNA levels were again analyzed by primer extension. The WT genome was also used in a parallel study to serve as a control. As shown in Fig. 5, the precore RNA and the core RNA levels of the WT genome were not significantly affected by HNF1. In contrast, HNF1 reduced in a dose-dependent manner the precore RNA level of the M1 double mutant to approximately 30% and in the meantime slightly increased its core RNA level. This observation indicates that HNF1 plays a negative role in the precore RNA expression. Furthermore, this effect of HNF1

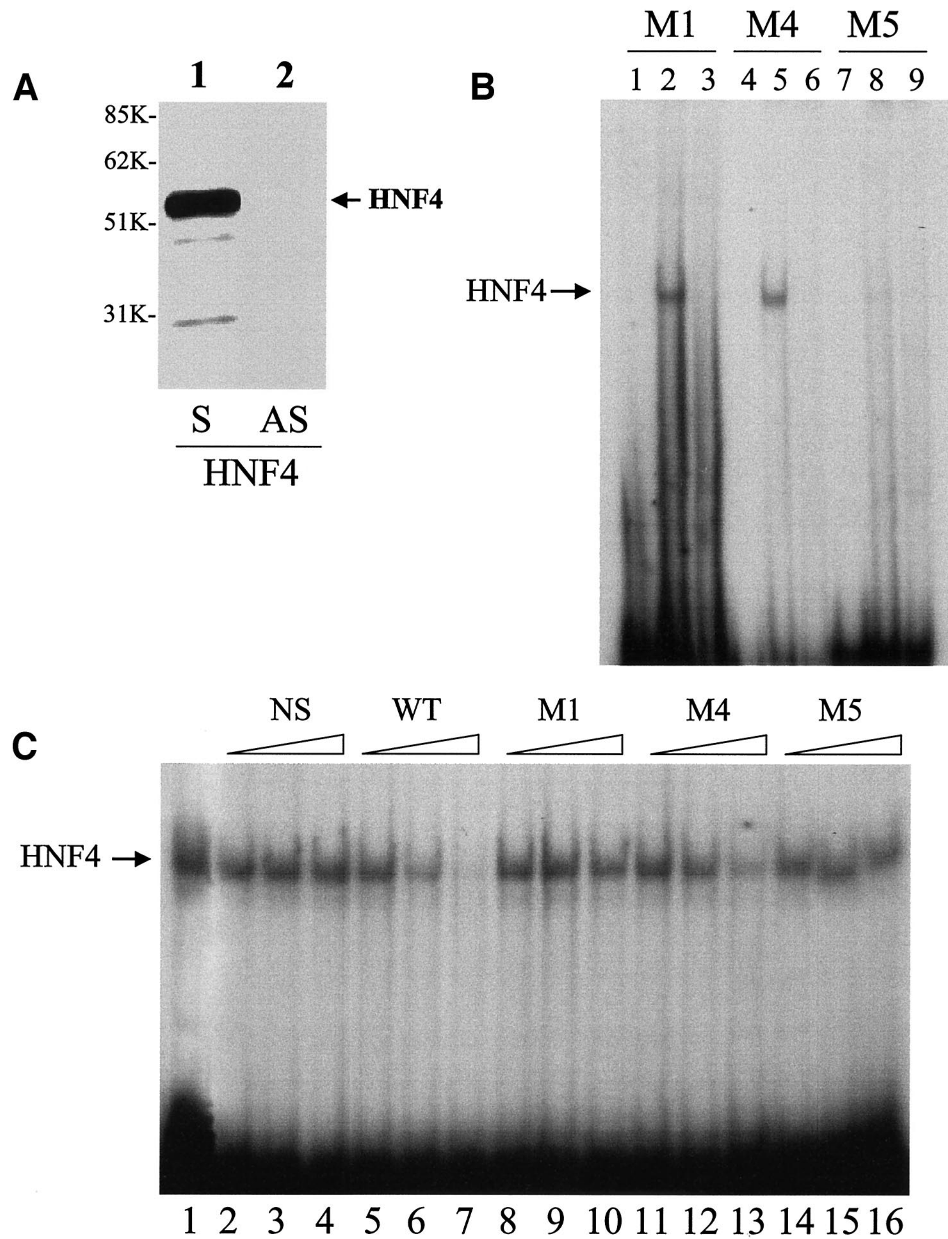

FIG. 3. EMSA for HNF4. (A) HNF4 was synthesized in vitro by using rabbit reticulocyte lysates and its sense RNA (lane 1) or antisense RNA (lane 2). The location of the HNF4 protein band is indicated by an arrow. (B) M1 (lanes 1 to 3), M4 (lanes 4 to 6), and M5 (lanes 7 to 9) DNA probes were used for the EMSA. Lanes 1, 4, and 7, free DNA probes; lanes 2, 5, and 8, the DNA probes were incubated with the translation mixture containing the HNF4 sense RNA; lanes 3, 6, and 9, the DNA probes were incubated with the translation mixture containing the antisense HNF4 RNA. The arrow indicates the location of the HNF4 band shift. (C) Competition assay. The EMSA was performed by using the WT DNA probe in the absence (lane 1) or presence of 1:1 (lanes 2, 5, 8, 11, and 14), 1:10 (lanes 3, 6, 9, 12, and 15), or 1:100 (lanes 4, 7, 10, 13, and 16) nonlabeled competitor, indicated above the gel. NS, nonspecific competitor (see Materials and Methods).

apparently requires the HNF1 binding site located in the core promoter of the M1 genome, since the WT genome, which lacks this HNF1 site, did not respond to HNF1.

As we have previously demonstrated that HNF1 can interact with the HBV X protein to regulate the core promoter activity, we have also analyzed whether the suppressive effect of HNF1 on the M1 core promoter requires the X protein. The WT and M1 genomes incapable of expressing the X protein were again cotransfected with the HNF1 expression plasmid into Huh7 cells. As shown in Fig. 6, HNF1 again reduced the precore RNA level of the M1 genome lacking the ability to express X (X-negative) to about 38% and modestly increased its core RNA level. It did not have any significant effect on the X-negative WT genome. These results indicated that the X protein was not required for the suppressive activity of HNF1 on the precore RNA expression.

Role of the X protein in the regulation of HBV core promoter activities. To further investigate the possible role of the

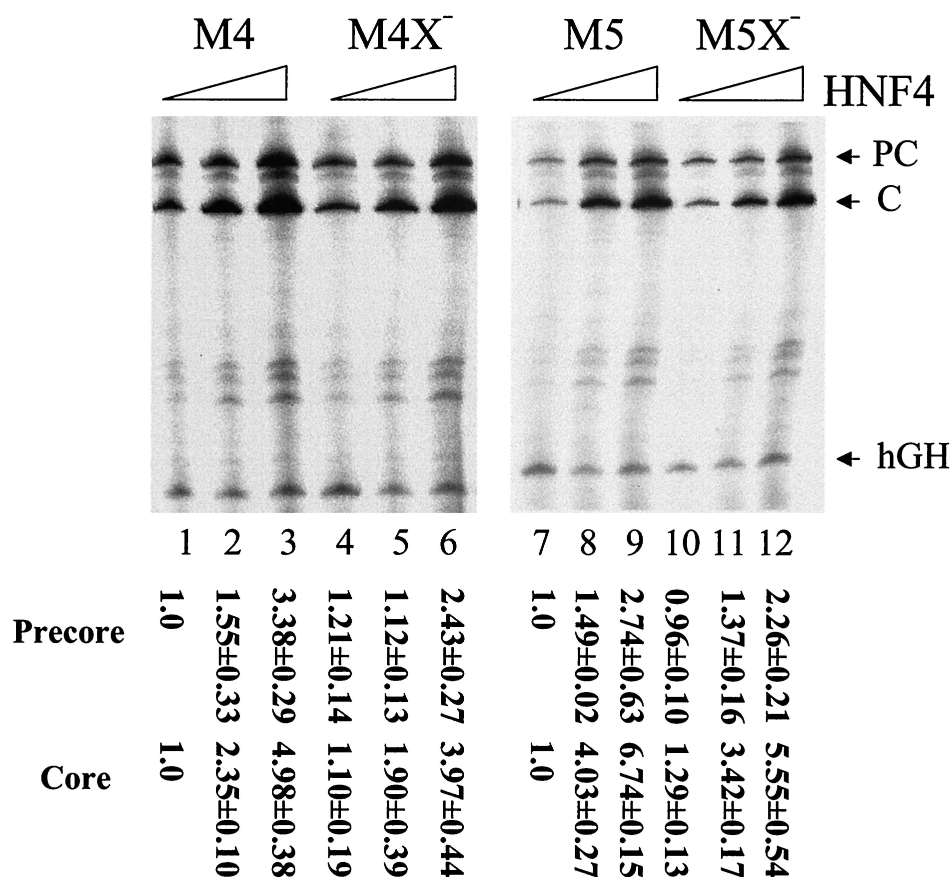

FIG. 4. Primer extension analysis of the precore RNA and the core RNA expressed from M4 and M5 DNA constructs. Huh7 cells were transfected with 4 μg of pM4D (lanes 1 to 3), pM4DX⁻ (lanes 4 to 6), pM5D (lanes 7 to 9), or pM5DX⁻ (lanes 10 to 12) HBV genomic DNA construct together with an increasing concentration of the HNF4 expression plasmid pLEN45 (18). Two micrograms of pXGH5 was also used for cotransfection to monitor the transfection efficiency. Lanes 1, 4, 7, and 10, no pLEN45 was used for cotransfection; lanes 2, 5, 8, and 11, 2 μg of pLEN45 was used; and lanes 3, 6, 9, and 12, 4 μg of pLEN45 was used. In these experiments, the control vector pLEN0 was used to bring the total amount of DNA used for the transfection to 10 μg. The results were quantified by SigmaScan Pro5 and normalized against the hGH RNA internal control as described in the legend to Fig. 2.

X protein, HNF4, and HNF1 in the regulation of the HBV core promoter activities, we compared the core promoter activities of WT, M1, M4, and M5 in the presence and absence of the X protein. These HBV genomic constructs, with and without the ability to express the X protein, were separately transfected into Huh7 cells. The expression of precore and core RNAs was then analyzed by primer extension. As shown in Fig. 7, abolishing the expression of the X protein did not significantly affect the core promoter activities of WT, M5, and M4 genomic constructs. However, it reduced the M1 core promoter activity, as evidenced by the nearly threefold reduction of the precore RNA level and the nearly twofold reduction of the core RNA level that were observed when M1X⁻ was compared with M1. This result is consistent with our previous observation that the X protein mutant can interact with HNF1 to regulate the M1 core promoter activities (13).

DISCUSSION

The natural HBV variant that carries the nt 1765 A-to-T and nt 1767 G-to-A double mutation is frequently isolated from patients with chronic hepatitis symptoms (1, 15). This variant expressed a reduced level of the precore RNA with a modest increase of viral replication rate (3, 19). The nt 1765 A-to-T and nt 1767 G-to-A double mutation resides in the nuclear receptor binding site of the core promoter and abolishes the binding of COUP-TFs, the heterodimer of PPARα-RXRα, and TR4 without affecting the binding by HNF4 (19). This double mutation also creates an HNF1 binding site and changes two codons in the overlapping X protein coding sequence. Thus, the suppressive effect on the precore RNA expression by this double mutation may be due to HNF4, HNF1, and/or the X protein. In this report, we have studied the possible effects of HNF1, HNF4, and the X protein on the core promoter activities. Our results indicate that HNF4 could stimulate the expression of the precore RNA and the core RNA from both the WT and the M1 double-mutant genomes, although its effect on the core promoter of the M1 genome was less prominent (Fig. 2). The HNF4 binding site in the core promoter is apparently not needed for the transactivation effect of HNF4, since the M5 core promoter, which lacks the HNF4 binding site, was also stimulated by HNF4 (Fig. 4). It is conceivable that HNF4 stimulates the core promoter through

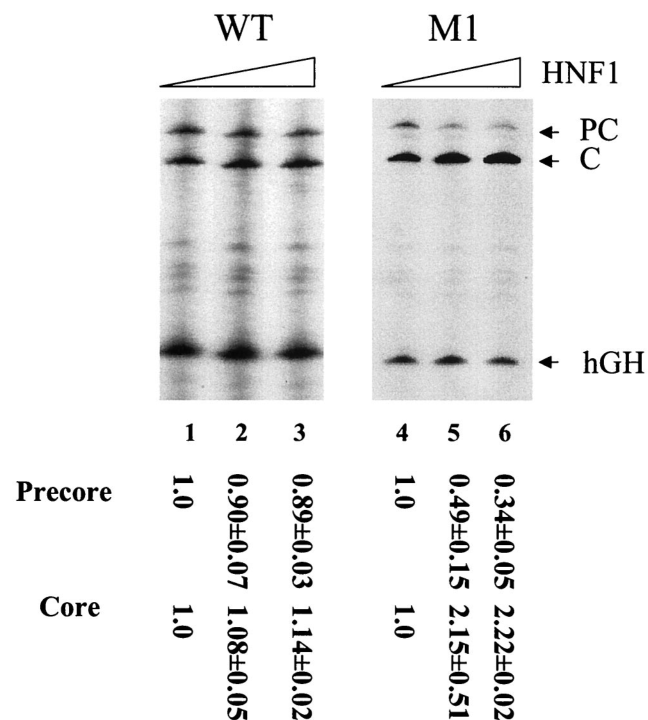

FIG. 5. Effects of HNF1 on the HBV core promoter. Huh7 cells were cotransfected with 4 μg of pWTD (lanes 1 to 3) or pM1D (lanes 4 to 6) HBV genome and various concentrations of the HNF1 expression plasmid pCMV-HNF1. Two micrograms of pXGH5 was also included in the cotransfection experiment to monitor the transfection efficiencies. Lanes 1 and 4, no pCMV-HNF1 was used for the cotransfection; lanes 2 and 5, 2 μg of pCMV-HNF1 was used; lanes 3 and 6, 4 μg of pCMV-HNF1 was used. The control vector pRc/CMV was used to bring the total amount of DNA to 10 μg for transfection. The locations of the precore RNA, the core RNA and the hGH RNA are indicated with arrows. The quantification was conducted as described in the legend to Fig. 2. The experiments were repeated at least three times and the results shown represent the averages ± standard errors of the experiments.

its binding site in the EN1 enhancer and/or the EN2 enhancer. Indeed, Yu and Mertz had previously reported that HNF4 could stimulate the core promoter via its binding site in the EN1 enhancer (24).

In contrast to the positive effect of HNF4, HNF1 suppressed the precore RNA expression from the M1 double-mutant core promoter and marginally increased the core RNA level (Fig. 5). The suppressive effect of HNF1 is apparently mediated by its binding site in the M1 core promoter, as HNF1 has no effect on the WT core promoter, which lacks the HNF1 binding site. Raney et al. (17) previously suggested that HNF4 binding to the nuclear receptor binding site of the M1 double mutant might limit the precore RNA synthesis. Based on the results of the present study, however, it is apparent that it is not HNF4 but HNF1 that is responsible for this suppression of the precore RNA synthesis. The suppressive effect of HNF1 on the precore RNA does not require the X protein, since abolishing the expression of the X protein did not abolish this suppressive effect (Fig. 6). Note that HNF1 did not completely abolish the expression of the precore RNA, which accounts for the low expression level of the e antigen by the M1 double mutant (1, 2, 19).

To investigate the role of the X protein in the regulation of the core promoter activities and to understand how it may interact with HNF1 and HNF4, we have studied the expression of precore and core RNAs from WT, M1, M4, and M5 genomic constructs, with and without the ability to express the X protein. Our results indicate that abolishing the expression of the X protein reduced the core promoter activity of only the M1 genomic construct (Fig. 7). This observed reduction of the M1 core promoter activity is consistent with a previous finding (13), which indicated that the X protein could interact with HNF1 physically and functionally to activate the M1 core promoter. The lack of effects of the X protein on WT, M4, and M5 core promoters indicates that the HNF1 binding site in the core promoter is required for the X protein to stimulate the core promoter activities.

In agreement with a previous report (11), the M4 genome expressed approximately two times more precore RNA and core RNA than did the M5 genome (Fig. 7). It had been previously postulated that this might be due to the M5 X protein (11), which differs from the M4 X protein by two amino acids due to the double mutation (Fig. 1). However, since the $M4X^-$ genome still expressed approximately two times more precore and core RNAs than did the $M5X^-$ genome, it does

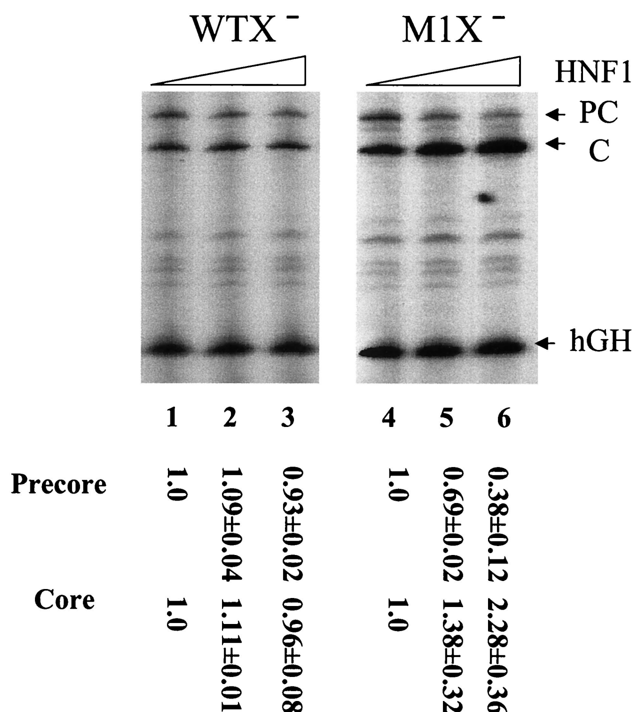

FIG. 6. Effects of HNF1 on the HBV core promoter in the absence of the X protein. The experiments were conducted as described in the legend to Fig. 5 except that $pWTDX^-$ (lanes 1 to 3) and $pM1DX^-$ (lanes 4 to 6) genomic constructs were used for the transfection studies. Lanes 1 and 4, no pCMV-HNF1 was used for the cotransfection; lanes 2 and 5, 2 μg of pCMV-HNF1 was used; lanes 3 and 6, 4 μg of pCMV-HNF1 was used. The control vector pRc/CMV was used to bring the total amount of DNA to 10 μg for transfection. The locations of the precore RNA, the core RNA, and the hGH RNA are indicated with arrows.

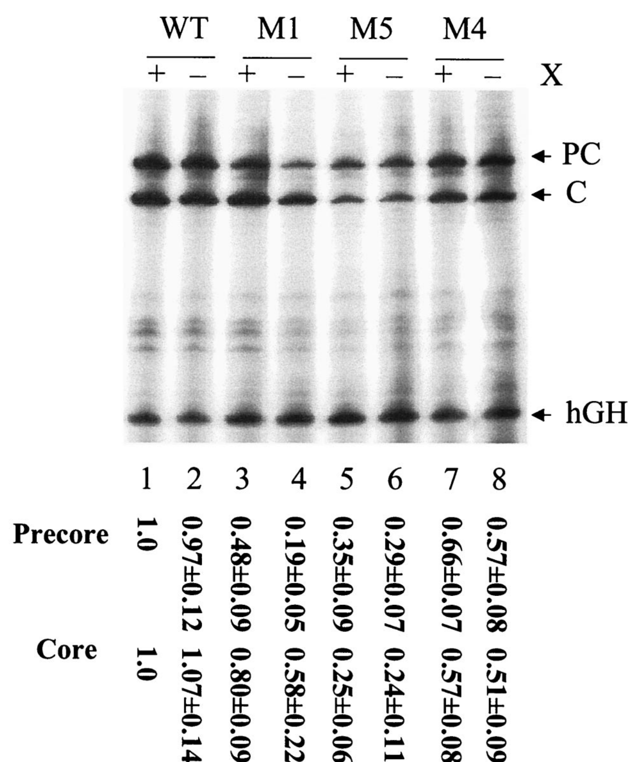

FIG. 7. Effects of the X protein on the HBV core promoter activities. HBV genomic constructs pWTD (lanes 1 and 2), pM1D (lanes 3 and 4), pM5D (lanes 5 and 6), and pM4D (lanes 7 and 8), with and without the ability to express the X protein were transfected into Huh7 cells. Forty-eight hours after transfection, cells were lysed for RNA isolation. The HBV precore and core RNAs were analyzed by primer extension as described in Materials and Methods. + and − indicate X-positive and X-negative genomes, respectively. Arrows indicate the locations of the precore RNA, the core RNA, and the hGH RNA bands. Quantification of the precore RNA and the core RNA signals was conducted as described in the legend to Fig. 2.

not appear likely that the mutated X protein was responsible for the reduction of the M5 core promoter activities. It is likely that the difference in their core promoter activities is due to the difference in their abilities to bind to nuclear receptors.

In conclusion, our results demonstrate that HNF4 can activate the WT precore and core RNA expression. It can also activate the M1 precore and core RNA expression, although the activation on the M1 precore RNA expression by HNF4 is antagonized by HNF1. The X protein does not affect the ratio of the M1 precore RNA and the core RNA, but it can increase the absolute amount of both RNAs. This three-way interaction on the regulation of the M1 core promoter leads to the specific suppression of the precore RNA expression, which we believe then alters the course of replication and pathogenesis of HBV in chronically infected patients.

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