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Complex Regulation of Transcription from the Hepatitis B Virus Major Surface Antigen Promoter in Human Hepatoma Cell Lines†

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A detailed mutational analysis of the regulatory DNA sequence elements that control expression of the hepatitis B virus major surface antigen gene was performed in the human hepatoma cell lines HepG2.1 and Huh7, using transient transfection assays. Seven regions (A to G) of the major surface antigen promoter located within 200 nucleotides of the RNA initiation site have been identified which influence the level of transcription from this promoter. The three distal regions (A to C), located between −188 and −68, appear to possess a level of redundancy in their ability to influence the transcriptional activity from the major surface antigen promoter. The simultaneous deletion of regions A, B, and C resulted in an approximately fourfold reduction in transcription from the major surface antigen promoter. Region D, located between −67 and −49, is an essential element of the major surface antigen promoter. The three proximal regions (E to G) are located within 45 nucleotides of the major transcription initiation site. Region E prevents the negative influence of region F and can compensate for the effect of mutation of region G on transcription from the major surface antigen promoter. Region G can compensate for the effect of the loss of a functional region E sequence on the transcriptional activity of the major surface antigen promoter only in the absence of a functional region F sequence. These results imply that the level of expression of the major surface antigen gene is controlled by the complex interplay between a minimum of six transcription factors which activate and one transcription factor which represses transcription from this gene.

The hepatitis B virion contains a circular 3.2-kb partially double stranded DNA genome which is packaged within a 27-nm nucleocapsid of hepatitis B virus (HBV) core antigen (21). The nucleocapsid is enveloped by a lipoprotein coat composed of cellular lipid and HBV surface antigen to produce the 42-nm virus particle (11, 31). The virus coat or envelope contains three surface antigen polypeptides, the major, middle, and large polypeptides, which are encoded by a single open reading frame which possesses three in-frame initiation codons (11, 14, 31). The major and middle surface antigen polypeptides are synthesized from abundant 2.1-kb HBV RNAs which are transcribed from the major surface antigen promoter (11, 17).

The regulatory sequence elements that control the expression of the 2.1-kb major surface antigen gene transcript have been analyzed in a variety of cell lines (1, 3, 6, 7, 10, 18, 20, 26, 27). The HBV enhancer sequence has been shown to influence the level of transcription from the major surface antigen promoter to different extents in a variety of transfection assay systems (1, 3, 6, 10), and the major surface antigen promoter has been located within 200 nucleotides of the transcription initiation sites of the 2.1-kb RNAs by deletion analysis (7, 20, 26). However, a detailed mutational analysis of this promoter has not been described.

After transfection with the complete HBV genome, HBV can be produced by certain human hepatoma cell lines (5, 25, 30, 32, 33), including Huh7 cells. Therefore, it appears likely that the transcriptional regulatory mechanisms which operate during infection of hepatocytes in vivo may be most precisely mimicked in these cell lines. For this reason, a detailed mutational analysis of the transcriptional regulatory sequence elements which control the level of transcription from the major surface antigen promoter was performed, using transient transfection assays in the differentiated human hepatoma cell line Huh7. For comparison, the activities of the mutant major surface antigen promoter constructs were also analyzed in the dedifferentiated human hepatoblastoma cell line HepG2.1 (19). This analysis indicated that multiple sequence elements which probably bind specific transcription factors regulate the level of expression from the major surface antigen promoter in a complex manner. However, the major surface antigen promoter appears to be regulated in a similar manner in both the differentiated Huh7 and the dedifferentiated HepG2.1 cells.

MATERIALS AND METHODS

Plasmid constructions. The various steps in cloning of the plasmid constructs used in the transfection experiments were performed by standard techniques (22). The HBV sequences in these constructions were derived from plasmid pCP10, which contains two copies of the HBV genome (subtype *ayw*) cloned into the *Eco*RI site of pBR322 (9). Plasmid SpLUC has been described previously (19). The unique HBV *Xho*I site used in this construct is located 157 nucleotides 3′ to a predominant surface antigen gene transcription initiation site (4, 20). Therefore, plasmid SpLUC contains one complete HBV genome located directly 5′ to the promoterless firefly luciferase (LUC) reporter gene such that expression of the LUC gene is governed by the HBV major surface antigen promoter. The plasmid constructs containing the various linker scanning (LS) mutations, insertions (IN), and deletions (Δ5′ and Δ3′/Δ5′) in the major surface antigen promoter (Fig. 1 to 6) were generated by appropriate restriction endonuclease and *Bal*31 nuclease

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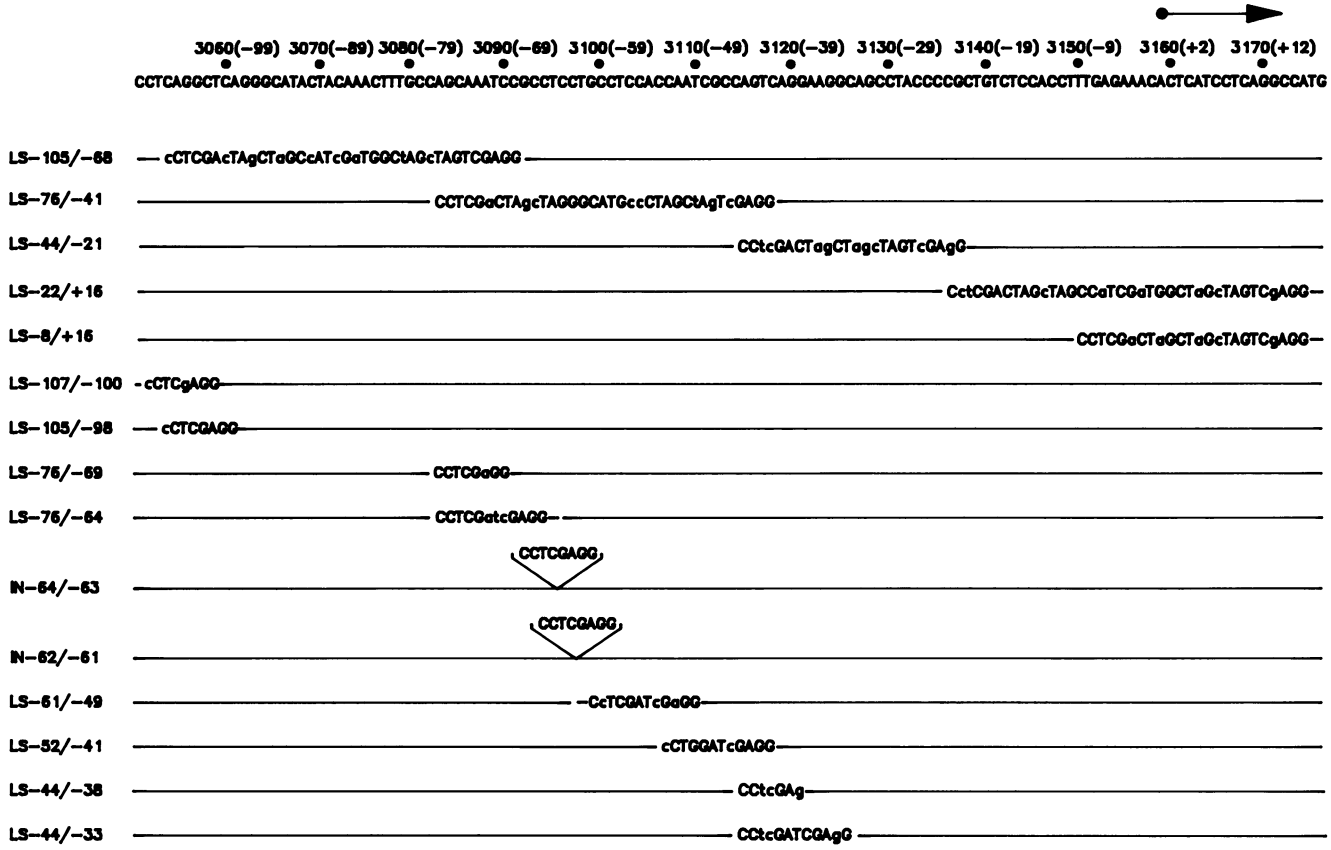

FIG. 1. Sequences of the major surface antigen promoter linker scanning mutations. The promoter sequence (subtype *ayw*) with nucleotide coordinates and transcription initiation site (arrow) is shown at the top. Coordinates of the major surface antigen promoter region are derived from the GenBank gene sequence data bank; their positions relative to a predominant transcription initiation site (+1, nucleotide coordinate 3159) are given in parentheses. Nucleotide substitutions in the linker sequences are indicated in uppercase; unchanged nucleotides are indicated in lowercase. Deletion of a nucleotide is indicated by a hyphen, and linker insertions are indicated above the line. The linker scanning mutations were introduced into construct Δ5′−188.

digestions of HBV sequences and subsequent cloning steps similar to those used to construct SpLUC. All deletion breakpoints generated by *Bal*31 nuclease digestion were determined, and linker scanning mutations were confirmed by dideoxynucleotide sequencing (23). The mutations (Fig. 1 to 6) in the major surface antigen promoter were constructed as previously described (13, 15), starting with the Δ5′−188 plasmid and using an *Xho*I linker (CCTCGAGG) to create the linker scanning mutations, insertions, or internal deletions. Linker scanning mutations larger than 8 nucleotides were produced by cloning additional linker sequences into specific internal deletion constructs. The Δ5′−188 plasmid contains the major surface antigen promoter region from −188 (coordinate 2971) to +157 (coordinate 133). A predominant transcription initiation site previously observed in transient transfection assays (20) has been designated +1 (coordinate 3159). The coordinates of the major surface antigen promoter region are derived from the GenBank gene sequence data bank.

Cells and transfections. The human hepatoma cell lines HepG2.1 and Huh7 were grown in RPMI 1640 medium and 10% fetal calf serum at 37°C in 5% CO_2-air and were transfected as previously described (16). The transfected DNA mixture consisted of 15 μg of a LUC plasmid and 1.5 μg of pSV2CAT (12), which served as an internal control for transfection efficiency. pSV2CAT directs expression of the chloramphenicol acetyltransferase (CAT) gene under the control of the simian virus 40 (SV40) early promoter. Cell extracts were prepared 40 to 48 h after transfection and assayed for LUC and CAT activities as previously reported (8).

RESULTS

Deletion analysis from the 5′ end of the HBV major surface antigen gene promoter. The transcriptional regulatory elements that control expression of the HBV major surface antigen gene were analyzed by using transient expression assays in the human hepatoma cell lines HepG2.1 and Huh7. A series of 5′ deletions was tested for its effect on the transcriptional activity of the major surface antigen promoter (Fig. 2). Transcriptional activity was not affected when sequences 5′ to −124 were deleted, indicating that maximal promoter activity in both cell lines examined can be supported by sequences between −124 and +157. In HepG2.1 cells, it appears that the sequences between −103 and −100 influence the activity of the surface antigen promoter, as there was approximately a fivefold reduction in relative LUC activity between constructs Δ5′−103 and Δ5′−99 (Fig. 2). This observation defines the 5′ boundary of

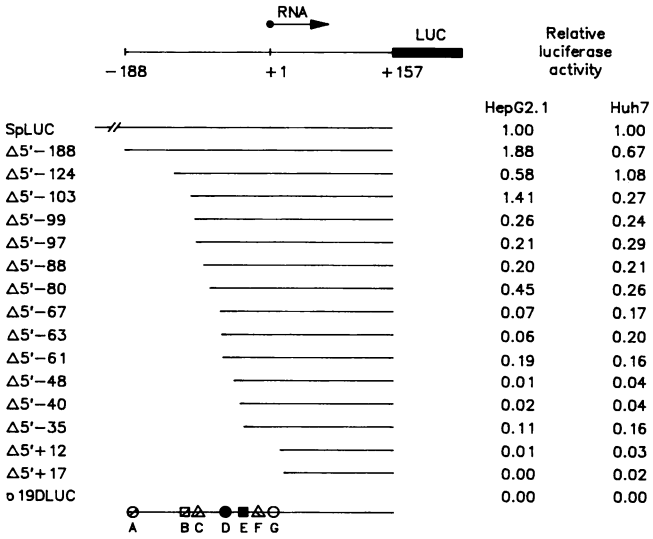

FIG. 2. Deletion analysis from the 5′ end of the HBV major surface antigen promoter. Construct SpLUC contains one complete HBV genome located directly 5′ to the promoterless LUC reporter gene such that expression of the LUC gene is governed by the major surface antigen promoter. Horizontal lines indicate the HBV sequences present in the various LUC constructs. The diagram at the top represents construct Δ5′−188 and indicates the 188 nucleotides of 5′ untranscribed sequence, the major transcription initiation site (+1), and the 157 nucleotides of transcribed HBV sequence present in this construct. Similarly, designations of the other 5′ deletions indicate the extent of the promoter region present in each construct. The diagram at the bottom indicates the proposed transcription factors involved in regulation of expression from the surface antigen promoter and the regions to which they bind (regions A to G). The internal control used to correct for transfection efficiencies was pSV2CAT.

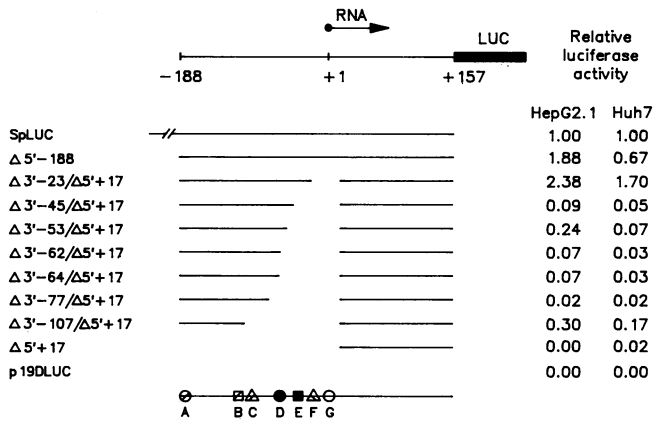

FIG. 3. Deletion analysis of Δ5′−188 from the 3′ end of the HBV major surface antigen promoter. Designations are as for Fig. 2. For internal deletions, the designations (13) indicate which sequences from 3′ deletion mutants (Δ3′) have been subcloned into a 5′ deletion mutant (Δ5′). Hence, construct Δ3′−23/Δ5′+17 lacks the promoter sequence between −22 and +16. The internal control used to correct for transfection efficiencies was pSV2CAT.

region C at −103 (Fig. 7). In Huh7 cells, it appears that the sequences between −124 and −104 influence the activity of the surface antigen promoter, as there was approximately a fourfold reduction in relative LUC activity between constructs Δ5′−124 and Δ5′−103 (Fig. 2). This observation defines the 5′ boundary of region B at or near −124 (Fig. 7).

In both cell lines, further analysis of this 5′ deletion series suggested that the sequences between −61 and −49 influence the activity of the surface antigen promoter, as there was a reduction in relative LUC activity between constructs Δ5′−61 and Δ5′−48 (Fig. 2). This observation indicated that the 5′ boundary of region D is located at or near −61 (Fig. 7). Further analysis of the surface antigen promoter supported this suggestion (Fig. 4 and 5). These results appear to differ from those previously reported for the analysis of a series of 5′ deletions of the major surface antigen promoter (7). This discrepancy may be due to the difference in the subtypes of the HBV DNA and the cell lines used in these analyses.

Deletion analysis from the 3′ end of the HBV major surface antigen gene promoter. A series of 3′ deletions of the Δ5′−188 construct was tested for its effect on the transcriptional activity of the major surface antigen promoter (Fig. 3). This construct was chosen for this and subsequent deletion and mutational analyses because it had previously been shown to contain all of the regulatory elements of the surface antigen promoter and all of the observed binding sites for the sequence-specific DNA-binding proteins which interact with this promoter (20). Transcriptional activity was not affected when sequences from −22 to +16 were deleted, indicating that maximal promoter activity in both cell lines examined can be supported by the sequences located 5′ to −22 (Fig. 3). However, it appears that the sequences between −44 and −23 influence the activity of the surface antigen promoter, as there was a greater than 10-fold reduction in relative LUC activity between constructs Δ3′−45/Δ5′+17 and Δ3′−23/Δ5′+17 (Fig. 3). This observation defines the 3′ boundary of region E at −23 (Fig. 7).

Analysis of the effects of internal deletions and large linker scanning mutations on transcription from the HBV major

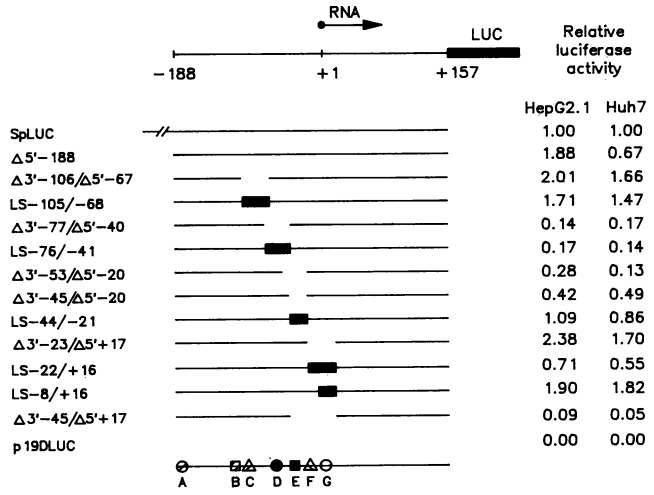

FIG. 4. Analysis of the effects of internal deletions and large linker scanning mutations on transcription from the HBV major surface antigen promoter. Designations (13, 15) are as for Fig. 3. For linker scanning mutations, the region of the promoter sequence replaced by linker sequences is indicated. Hence, construct LS−105/−68 contains linker sequences between −105 and −68. Boxes indicate the locations of the linker scanning mutations. The internal control used to correct for transfection efficiencies was pSV2CAT.

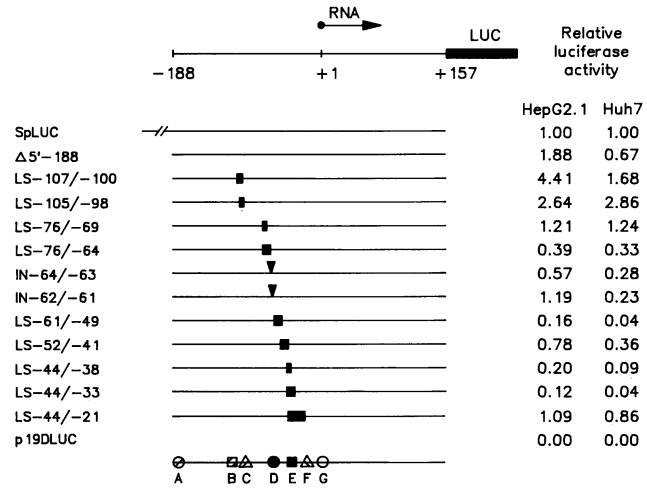

FIG. 5. Analysis of the effects of small linker scanning mutations on transcription from the HBV major surface antigen promoter. Designations are as for Fig. 4. Triangles indicate locations of the linker insertions. For linker insertions, the designation indicates the nucleotides between which the insertion occurs. The internal control used to correct for transfection efficiencies was pSV2CAT.

surface antigen gene promoter. A series of related internal deletions and large linker scanning mutations (Fig. 1) was introduced into the Δ5′−188 construct, and the effect on the transcriptional activity of the major surface antigen promoter was examined (Fig. 4). Transcriptional activity was not affected when sequences from −105 to −68 (region C) were deleted (Δ3′−106/Δ5′−67) or mutated (LS−105/−68), indicating that maximal promoter activity in both cell lines examined can be supported without these nucleotides. This observation has two major implications. First, it demonstrates that the 5′ boundary of region D is located 3′ to −68 (Fig. 7). This is consistent with the results for the 5′ deletion series (Fig. 2). Second, it indicated that in HepG2.1 cells the loss of region C can be compensated for by sequences located between −188 and −105 (Fig. 2 and 4).

The reduction of transcriptional activity observed with constructs Δ3′−77/Δ5′−40 and LS−76/−41 indicates that the deletion or mutation of region D results in an approximately 5- to 10-fold reduction in transcription from the surface antigen promoter (Fig. 4). The observation that

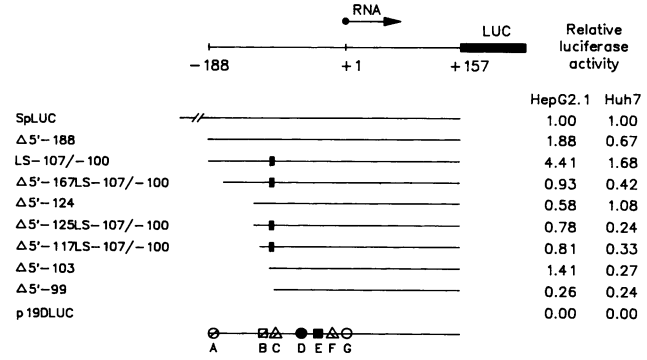

FIG. 6. Deletion analysis from the 5′ end of construct LS−107/−100. Designations are as for Fig. 4. The internal control used to correct for transfection efficiencies was pSV2CAT.

constructs Δ3′−45/Δ5′−20 and LS−44/−21 display essentially full surface antigen promoter activity establishes that the 3′ boundary of region D does not extend past −45 (Fig. 4). However, the greater than fivefold reduction in the level of surface antigen promoter activity observed from construct Δ3′−53/Δ5′−20 is consistent with region D extending further than −53 (Fig. 4).

From the 3′ deletion constructs Δ3′−23/Δ5′+17 and Δ3′−45/Δ5′+17 (Fig. 3 and 4), it was apparent that the sequence from −44 to −23 contains an essential sequence element of region E required for maximal promoter activity in the absence of sequences from −22 to +16. Therefore, it was surprising that constructs Δ3′−45/Δ5′−20 and LS−44/−21, which are deleted or mutated between −44 and −21, showed no reduction in surface antigen promoter activity (Fig. 4). In addition, constructs Δ3′−23/Δ5′+17 and LS−22/+16, which are deleted or mutated between −22 and +16, displayed full surface antigen promoter activity, whereas construct Δ3′−45/Δ5′+17 displayed minimal promoter activity (Fig. 4). This finding showed that the sequence between −44 and +16 is essential for maximal transcriptional activity but that the separate deletion or mutation of the sequence between −44 and −21 (region E) or −22 and +16 (region G) did not affect the transcriptional activity of the major surface antigen promoter. This implies that there is a regulatory sequence element located 3′ to −21 (region G) which can compensate for the deletion or mutation of sequences from −44 to −21 (region E), indicating that functionally the transcription factors which bind to regions E and G can substitute for each other but that their influence on the transcriptional activity of the surface antigen promoter is not additive.

Analysis of the effects of short linker scanning mutations on transcription from the HBV major surface antigen gene promoter. A series of short linker scanning mutations (Fig. 1) was introduced into construct Δ5′−188, and the effect on the transcriptional activity of the major surface antigen promoter was examined (Fig. 5). Transcriptional activity was not affected when the linker scanning mutations in constructs LS−107/−100, LS−105/−98, and LS−76/−69 were examined (Fig. 5), indicating that maximal promoter activity in both cell lines can be supported without the nucleotides that were mutated in these constructs. However, the linker scanning mutation in construct LS−76/−64 resulted in about a threefold reduction in transcriptional activity from the surface antigen promoter (Fig. 5). The influences of mutations LS−76/−69 and LS−76/−64 position the 5′ boundary of region D between −68 and −64, consistent with the positioning of the 5′ boundary of region D 3′ to −68 as determined from the absence of any effect of mutation LS−105/−68 (Fig. 4). In addition, it is noteworthy that mutations LS−76/−64, IN−64/−63, and IN−62/−61 all have a relatively small effect on the transcriptional activity of the surface antigen promoter (Fig. 5). This finding suggests that the sequence between −67 and −62 only moderately reduces transcription from the surface antigen promoter. In contrast, mutation LS−61/−49 results in an approximately 10-fold reduction in transcriptional activity from the surface antigen promoter (Fig. 5). This finding indicates that the most critical sequence in region D is located between −61 and −52, since mutation LS−52/−41 reduces the transcriptional activity of the surface antigen promoter only two- to threefold (Fig. 5). This moderate reduction in transcriptional activity observed with mutation LS−52/−41 is probably due to minor influences on the functionally important sequences of regions D and/or E and

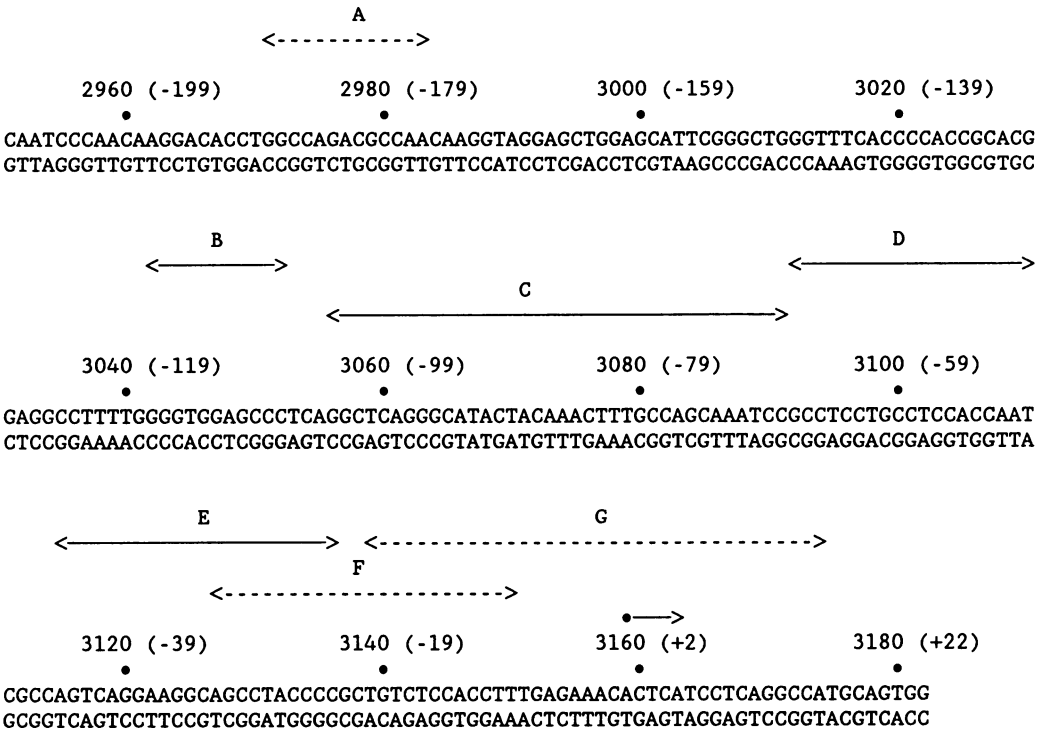

FIG. 7. Sequence of the HBV major surface antigen promoter region (subtype *ayw*). The nucleotide coordinates are shown (●); an arrow indicates the approximate location of a predominant HBV major surface antigen transcription initiation site (+1, nucleotide coordinate 3159) (20). The coordinates of the major surface antigen promoter region are derived from the GenBank gene sequence data bank; their positions relative to a predominant transcription initiation site are given in parentheses. The functionally important regulatory sequence elements of the promoter are identified (regions A to G). The limits of regions B to E have been precisely defined and are indicated with solid arrows. Regions A, F, and G have been less well defined, and their approximate (regions F and G) or most probable (region A) locations are indicated with broken arrows (see text for explanation).

therefore defines the boundary between these two regions. These observations suggest that the functionally important sequences of region D do not extend beyond −49, and therefore region D comprises the sequence between −67 and −49.

The linker scanning mutations in constructs LS−52/−41, LS−44/−38, and LS−44/−33 indicate that the 5′ boundary of region E is located 3′ to −45. This conclusion is based on the observation that mutation LS−52/−41 only modestly influences the transcriptional activity of the surface antigen promoter, whereas mutations LS−44/−38 and LS−44/−33 reduce the promoter activity greater than 10-fold. Since the 3′ deletion series of the surface antigen promoter (Fig. 3) has mapped the 3′ end of region E 5′ to −22, this locates the functional sequences defining region E between −44 and −23.

An additional complexity in the regulation of surface antigen gene expression is apparent when the transcriptional activities of constructs LS−44/−38 and LS−44/−33 (Fig. 5) are compared with those of constructs Δ3′−45/Δ5′−20 (Fig. 4) and LS−44/−21 (Fig. 4 and 5). Analysis of these constructs shows that deletion or mutation of sequences from −44 to −21 does not influence the transcriptional activity of the surface antigen promoter. However, mutations LS−44/−38 and LS−44/−33, which alter the sequences from −44 to −33, display dramatically reduced promoter activities. These observations imply that mutation of a functional region E permits a negative regulatory element located in part between −32 and −21 to function and suppress the activity of the surface antigen promoter. They also demonstrate that the 5′ end of region F is located 3′ to −33 (Fig. 7). In addition, these observations suggest that the positive influence on transcription mediated through region E prevents the action of the negative influence mediated through region F, whereas the positive influence mediated through region G, which can compensate for the loss of the positive influence of region E on transcription, can do so only in the absence of a functional region F (Fig. 4 and 5). This finding indicates that the positive effect on transcription mediated through region E is dominant over the negative effect of region F, whereas the positive effect mediated through region G is not.

Deletion analysis from the 5′ end of construct LS−107/−100. Since it appeared that the most distal regulatory sequences in the surface antigen promoter might be utilized in a cell-type-specific manner (Fig. 2), this region of the promoter was analyzed further. A series of 5′ deletions of the construct LS−107/−100, which is mutated in region C, was tested for its effect on the transcriptional activity of the major surface antigen promoter (Fig. 6). In HepG2.1 cells, deletion of sequences to −117 (Δ5′−117LS−107/−100) resulted in no loss of transcriptional activity from the surface antigen promoter (Fig. 6). This finding indicates that the sequence from −117 to −107, representing region B, can compensate for the absence of a functional region C in HepG2.1 cells. This observation implies that the sequence-specific transcription factors which bind regions B and C display functional redundancy.

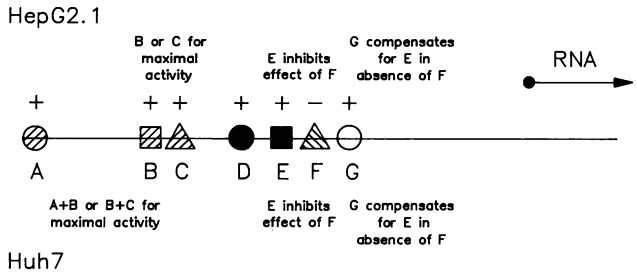

FIG. 8. Diagrammatic representation of transcription factors A to G, which regulate expression from the HBV major surface antigen promoter in HepG2.1 and Huh7 cells. Signs above the transcription factors indicate whether the factors positively (+) or negatively (−) regulate promoter activity.

In contrast, in Huh7 cells the 5′ deletion series of construct LS−107/−100 indicated that maximal surface antigen promoter activity was obtained only when sequences extending to −188 were present (LS−107/−100) (Fig. 6), despite the observation that region C had not been implicated in the regulation of this promoter in this cell line (Fig. 2 to 5). This finding suggested that one or more sequence elements (region A) located 5′ to region B were compensating for the loss of a functional region C. The precise localization of the functionally important sequence(s) within region A has not been achieved; however, the sequences between −188 and −126 include the important region, as construct LS−107/−100 has full promoter activity whereas construct Δ5′−125LS−107/−100 displays three- to fourfold-lower activity. These results indicate that maximal surface antigen promoter activity in Huh7 cells requires the presence of either region A plus region B (LS−107/−100) or region B plus region C (Δ5′−124) (Fig. 6). Unlike the case of HepG2.1 cells, maximal surface antigen promoter activity was not observed in Huh7 cells when the distal promoter region comprised only a functional region B (Δ5′−117LS−107/−100) or region C (Δ5′−103) (Fig. 6). However, deletion of regions A to C reduces the activity of the surface antigen promoter only three- to fourfold. This relatively small reduction in activity cannot easily be attributed to the influence of a specific promoter region. Therefore, the apparent differences observed in the regulation of the surface antigen promoter by these distal elements in HepG2.1 and Huh7 cells may reflect difficulties in measuring small changes in promoter activity rather than major differences in promoter regulation.

DISCUSSION

A detailed mutational analysis of the HBV major surface antigen promoter has been performed to determine the regulatory sequence elements that control the transcription of this gene. Seven regions (A to G) of the surface antigen promoter located within 200 nucleotides of the major transcription initiation site have been identified which modulate the level of its expression (Fig. 7). These regulatory sequence elements probably mediate their effects by interacting with sequence-specific DNA-binding transcription factors (Fig. 8) (20). The three distal regulatory elements (regions A to C) positively modulate the promoter activity approximately fourfold. It appears that maximal activity of the surface antigen promoter is observed in the dedifferentiated human hepatoma cell line HepG2.1 in the presence of either region B or region C. This finding suggests functional redundancy in the role of transcription factors B and C (Fig. 8), which bind to these regulatory sequence elements. Similarly, in the differentiated hepatoma cell line Huh7, maximal activity of the surface antigen promoter was observed in variously mutated promoter constructs. In this case, maximal transcriptional activity was observed when regions A plus B or B plus C were present in the promoter (Fig. 7 and 8). Considering the limited influence that the distal region has on the transcriptional activity of the surface antigen promoter and the difficulties involved in measuring small changes in promoter strengths, it seems appropriate to consider the distal regulatory sequences as comprising three regions capable of binding transcription factors (Fig. 8; transcription factors A to C) which display a degree of redundancy in their influence on the transcriptional activity of the surface antigen promoter. The exact role of each region in the regulation of this promoter will require more precise and extensive characterization of these regulatory regions and the transcription factors that interact with them.

The localization of the three distal regulatory regions has been variably established. Region A is poorly defined on the basis of this functional analysis and is localized between −188 and −126 (Fig. 7). Previously, it has been shown that purified nuclear factor 1 (CTF/NF1 [24]) and a sequence-specific DNA-binding protein from HepG2 cells bind to this region (−188 to −176; footprint region I) of the surface antigen promoter (20, 26). Therefore, it is possible that a transcription factor which is responsible for increasing the level of transcription from the surface antigen promoter through region A is NF1. Region B was precisely defined in HepG2.1 cells and shown to be located between −117 and −107 (Fig. 7). This region also binds a sequence-specific DNA-binding protein present in HepG2 cells (footprint region II [20]), suggesting that it may be this or a protein with similar DNA-binding specificity which is responsible for modulating the activity of the surface antigen promoter through this regulatory element. From the analysis of the surface antigen promoter in Huh7 cells, region B is less well defined, with its 5′ end being localized at or near −124 (Fig. 2). However, this observation does not exclude the possibility that it is the same transcription factor(s) which modulates the activity of the surface antigen promoter in both cell lines. Region C is located between −103 and −68. In HepG2 cells, there is a sequence-specific DNA-binding protein which binds to this region (footprint region III [20]), and it is possible that this transcription factor(s) (Fig. 8; transcription factor C) modulates the activity of the surface antigen promoter. The identities of transcription factors B and C are currently unknown since their recognition sequences do not correspond to those of any known transcription factors.

Region D is located between −67 and −49 and binds a transcription factor(s) (Fig. 8; transcription factor D) that is essential for maximal surface antigen promoter activity. This region displays a degree of homology to the SV40 origin of replication (4), although the significance of this observation is unclear. Region E is located between −44 and −23 and binds a transcription factor(s) (Fig. 8; transcription factor E) that prevents the negative influence of transcription factor F from reducing the transcriptional activity of the surface antigen promoter. Since region F is located 3′ to −32, it is apparent that the binding sites for transcription factors E and F may overlap, and therefore transcription factor E may prevent the action of transcription factor F by sterically preventing its binding to the surface antigen promoter. This could explain why transcription factor G, which binds to

region G located 3′ of −21, cannot prevent the negative influence of transcription factor F but has the capacity to compensate for the effect of the loss of transcription factor E on the transcriptional activity of the surface antigen promoter. The recognition sequences of transcription factors E and F do not resemble those of known transcription factors. However, the previously characterized binding site (footprint region IV) for a sequence-specific DNA-binding protein present in HepG2 cells (20) is located within region G. This recognition sequence (CTCATCCT; +2 to +9) is closely related to the binding site for a transcriptional control element (CTCANTCT), the initiator (28, 29), which has been identified at the transcription initiation site of many genes (28). Therefore, it is possible that transcription factor G is the initiator or a related protein. Regions E to G also display homology with the SV40 major late promoter (4), suggesting that the surface antigen promoter may be regulated in part by transcription factors arranged similarly to those regulating the SV40 late promoter (2).

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