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The hepatitis B virus: general description, physical structure, genetic organization, gene transcripts and genomic regulatory elements

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The HBV genome is a circular partially double-stranded DNA molecule. It contains four overlapping open reading frames (ORFs) genes. The S (preS1, preS2) region(s) encodes the major (small), middle and large proteins (HBsAg). The C and pre-C regions encode HBcAg and HBeAg. The X region encodes a polypeptide expressed during HBV infection. The P region codes for a protein with several functions in replication. Four classes of HBV mRNAs have been identified. In the HBV genome the pre-S1 promoter expresses the large protein. The pre-S2/S promoters produce both major and middle proteins. The X promoter produces 0.7 and 0.9 kb transcripts. The C and pre-C promoters produce the Core, pol(HBcAg), and the HBeAg proteins. Enhancer I and II are key regulatory elements in the transcriptional regulation of HBV. The activity of enhancer II is highly the liver specific. Enhancer II activates the transcriptional activity of both the pre-S1 and preS2/S promoters. Two HBV enhancers strongly affect the activity of all three major HBV promoters. A box-α in the II-A and box-β in II-B elements of HBV genome, are necessary for the enhancer II function. Either box-α or box-β can regulate the activity of the Core promoter, a, b, f proteins and c, d proteins bind to box-α and box-β, respectively, and mediate the enhancer function.

The study of human HBV which is one of the smallest known for animal virus, has been severaly limited because it only infects humans and because a tissue culture system in which this virus can be propagated is not available. (HBV can not be propagated *in vitro*). The hepatitis B surface antigen (HBsAg) transcription has been studied only in cell lines containing HBV DNA integrated into chromosomes, and HBsAg-related mRNAs 2.0 to 2.5 kb long have been described [1—5].

The hepatitis B viruses, also called *Hepadnaviruses* [6], represent a small group of primarily hepatotropic enveloped DNA viruses that proceed through reverse transcription of a RNA intermediate [7, 8] in a manner analogous to that of retroviruses.

Beside the HBV of man [9], this family (*Hapadnaviridae*) includes woodchuck hepatitis virus (WHV) of *Marmota monax* [10], ground squirrel hepatitis B virus (GSHV) of *Spermophilus beecheyi* [11], the tree squirrel hepatitis B virus [12], duck hepatitis B virus (DHBV) of *Anas domesticus* [13] and other ducks, heron hepatitis B virus in gray herons [14] and probably others. This taxonomy is derived from the relative hepatotropism of virus family members, their common virion morphology, genome size, structure and organization, and common mechanism of genome replication. All the viruses exhibit a strict host specificity; the human virus replicating only in man and a small number of higher apes.

The disease state induced by infection with HBV is manifest in varying ways characterized by the extent of liver inflammation and damage and viral persistence. In a small percentage of cases, primary infection leads to fulminant hepatitis resulting in severe liver dysfunction with very high mortality. Primary infection is most often resolved by complete clearance of the virus and development of immune memory to counter reinfection, but 5—10 % of infected adults develop chronic infection characterized by the persistence of viral antigens in the serum and accompanied by varying degrees of hepatic injury. This disease state may continue after integration of HBV DNA into the hepatocyte genome from which

transcription of viral antigen genes may continue in the absence of virion production. HBV can be detected in the hepatocyte either as a free DNA molecules or in an integrated form [15]. Chronically infected patients are predisposed to developing hepatocellular carcinoma (HCC) [16—19] with more than 100-fold greater probability than non-infected individuals [20]. The presence of integrated HBV DNA in hepatocellular carcinoma has led to the hypothesis that viral integration may contribute to the process of hepatocarcinogenesis [21, 22]. In hepatocytes that have undergone malignant transformation, part of HBV DNA are integrated into chromosomes of the host [23—31].

However, the mechanisms of integration, responsible for tumorigenesis and for the shutdown of HBV gene expression and replication in hepatocellular carcinoma remain unclear [32], but the integrated HBV subgenomes are suspected to be carcinogenic [3, 33—37]. Hepatitis B constitutes a major worldwide health problem with the number of chronically infected people currently estimated in excess of 250 million [38—40].

HBV has a partially double stranded, open-circular genome of 3.2 kb which contains four open reading frames (ORFs) including the S-gene encoding the surface antigen (HBsAg) and the C-gene encoding the Core antigen (HBcAg) (Fig. 1). A large ORF encompassing most of the viral genome encodes the viral polymerase while the fourth ORF encodes a protein of 154-amino acid residues which has been termed the X antigen (HBAg). Because the function of this product in the viral life cycle is still under intensive investigation.

Although the viral genome and RNA transcripts can be detected in extra-hepatic tissues of HBV-infected chimpanzees and in transgenic mice carrying the HBV DNA [39, 41—43], liver is still the principal site of clinical disease in which HBV actively replicates.

Infected plasma contains viral particles of different sizes and forms. During infection of humans, the virus (Dane particle) and the two prominent subviral particles (filaments and 22-nm particles) are observed in the sera of infected individuals. The virion has a diameter of approximately 42 nm (Dane particle) with a 27-nm core. The 22 nm diameter particles consist of empty viral envelopes that bears the hepatitis B surface antigen (HBsAg). Filaments and 22 nm particles consist only of the HBsAg and cellular lipid. Careful examination of the surface antigen [44, 45] has indicated that it is composed of at least three pairs of proteins: p24 and gp27; gp33 and gp36; p39 and gp42 (p: protein, gp: glycoprotein), where the second protein in each pair is a glycosylated from of the first. It is apparent from the work of a number of investigators [5, 31, 46] that these proteins are derived from a large open reading frame (one of ORFs) and originate from the first three strongly conserved ATGs in that region. This particular ORF consists of an «S» region that is preceded by an in-phase reading frame, which has been designated as «pre-surface» or «pre-S». The pre-S region may be further subdivided into «pre-S1» and «pre-S2» [46—48]. The 42 nm HBV particles (Dane particle = virion) contains three different surface proteins which are referred to as «major (small)» protein, «middle» protein and «large» protein. (The outer envelope of the virion is arrayed by three surface S proteins; the major (small) S, the middle S, and the large S [17, 44, 49, 50—53]). The large surface protein (LS) is translated from the first ATG codon of the surface open reading frame, while the middle and small (major) forms are translated from in-frame ATG codons further downstream. Inside the viral envelope, there is the 27-nm «Core» particle formed by subunits of core proteins referred to as HBcAg. It contains the viral polymerase and the partially double stranded DNA molecule to which a protein is covalently linked (for a review see [54]). All three forms of the surface (envelope) proteins (antigens) which are co-linear in the carboxyl-terminal protein, are needed for virion production and cotranslationally inserted into the Endoplasmic Reticulum (ER) as transmembrane proteins and, together with envelope cytoplasmic core (nucleocapsid) particles, form mature virions that are secreted via the constitutive secretory pathway [53], but an overproduction of the large form can result abnormal particle formation that becomes inspissated in the ER and damages the host cell [55, 56]. In addition, the middle and/or small forms, in the absence of other viral proteins, can bud into the lumen and be secreted in the form of spherical and filamentous subviral particles. LS, in contrast cannot be secreted when expressed alone but instead is retained within the cell in the form of intraluminal particles [55, 57, 58—60, 102].

If LS is coexpressed with the other forms of surface protein, they form heteromultimers whose phenotype depends on the relative amounts of the various surface proteins: a small relative amount of LS results in secretion, while a large amount results in retention. This retention affects the secretion not only of noninfectious subviral particles but also of the infectious virion particles and, therefore, is deleterious to the viral life cycle [61]. Not surprisingly, in the infected cell, LS is usually synthesized in much

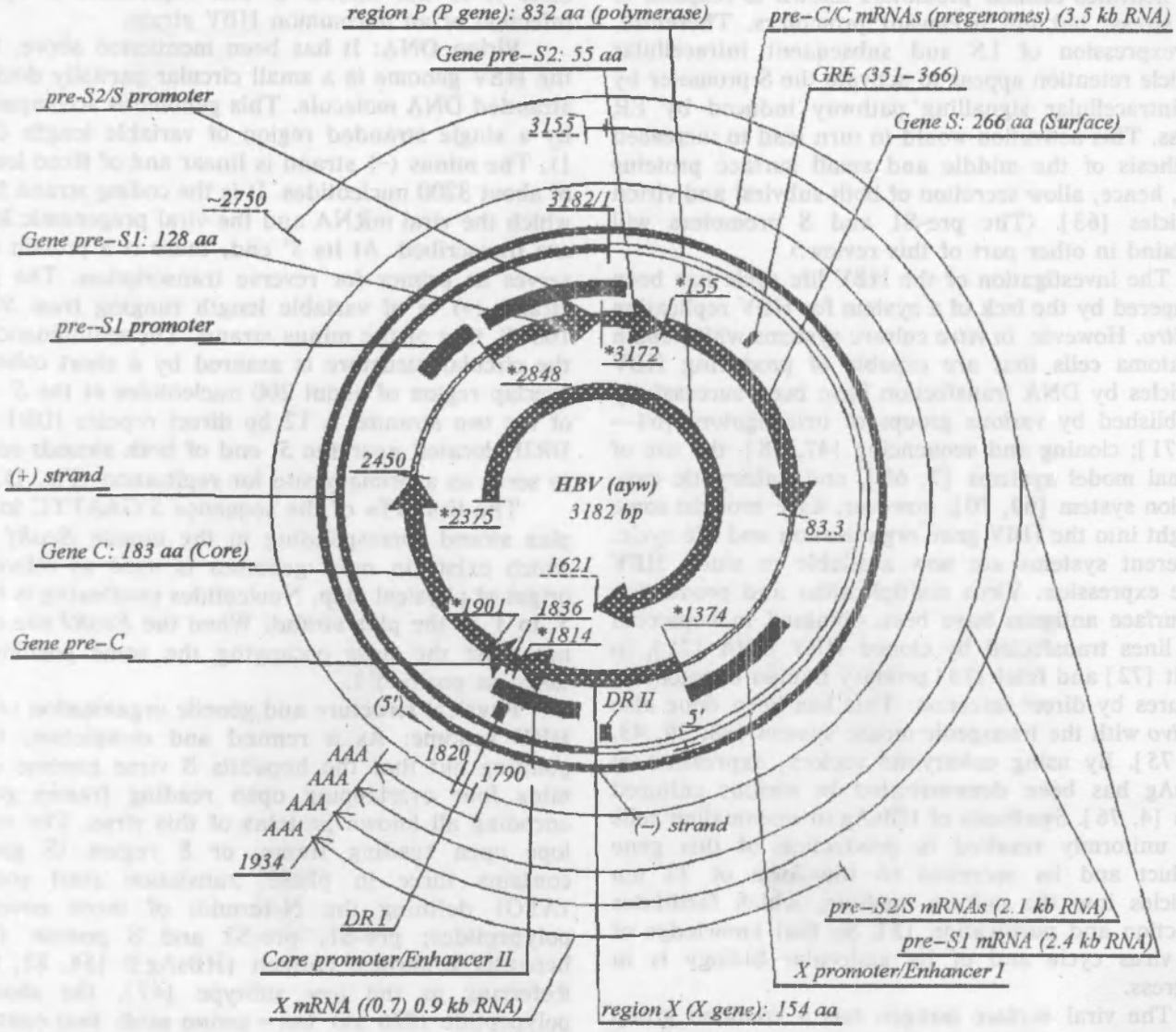

Fig. 1. HBV genome organization and viral transcripts (in *ayw* subtype): Open reading frames are represented by arrows, and boxes represented transcriptional control regions. The stars correspond to the ATG codons and the position given at the end of the arrows correspond to those of the stop codons. Abbreviation: GRE – Glucocorticoid Responsive Element, DRI and DRII – primary site for replication

smaller amounts than the middle and small surface proteins. This differential regulation is achieved by the presence of two independent promoters [62]. The upstream pre-S1 promoter gives rise to transcripts that are translated mainly into LS, while the downstream S promoter gives rise to transcripts capable of translation into only the middle and small surface protein. Since the amount of pre-S1 mRNA is normally much smaller than the amount of S mRNA, there is insufficient LS to prevent secretion. Because the relative ratio of LS to the middle and small surface proteins synthesized by HBV is crucial to its replication, it was found, that there is a feedback mechanism to ensure a balanced synthesis of these proteins, in that LS significantly activates the S promoter [63]. This activation is correlated whit the intracellular retention of LS and is mimicked by agents that induce ER stress [63]. Conversely, LS

also activates cellular promoters known to respond to ER stress, but not irrelevant promoters. Therefore, overexpression of LS and subsequent intracellular particle retention appear to activate the S promoter by an intracellular signalling pathway induced by ER stress. This activation would in turn lead to increased synthesis of the middle and small surface proteins and, hence, allow secretion of both subviral and virion particles [63]. (The pre-S1 and S promoters will explaind in other part of this review.)

The investigation of the HBV life cycle has been hampered by the lack of a system for HBV replication *in vitro*. However, *in vitro* culture systems whit human hepatoma cells that are capable of producing HBV particles by DNA transfection have been successfully established by various groups of investigators [64—67, 71]; cloning and sequencing [47, 48], the use of animal model systems [7, 68], and eukaryotic expression system [69, 70], however, have brought some insight into the HBV gene organization and life cycle. Different systems are now available to study HBV gene expression. Virus multiplication and production of surface antigens have been obtained in hepatoma cell lines transfected by cloned HBV DNA [71], in adult [72] and fetal [73] primary human hepatocytes cultures by direct infection. This has been done also *in vivo* with the transgenic mouse system [38, 39, 43, 74, 75]. By using eukaryotic vectors, expression of HBsAg has been demonstrated in various cultured cells [4, 76]. Synthesis of HBsAg in mammalian cells has uniformly resulted in production of this gene product and its secretion in the form of 22 nm particles into the culture medium, which facilitates detection and purification [5]. So that knowledge of the virus cycle and of its molecular biology is in progress.

The viral surface antigen has a common group specific determinant *a* and carries one member of each of the two pairs of mutually exclusive subtype determinants *d* and *y* [68] and *w* and *r* [78]. Thus there are four major subtypes of HBsAg: *adw, adr, ayw*, and *ayr* [79]. These subtypes have been recently classificated into group A, B, C and D by Okamoto et al. [80]. The groups A, B and D are homogenous while group C is not [80]. In this later group the *adw* genome is closely related to the *ayr* and *adr* genomes. Two by two analysis of the nucleotide sequences show some degree of divergence. The divergence is about 10 % for viruses of different subtypes and about 2 % for viruses of the same subtype except for the *ayr* subtype which diverges only 2 % from the *adr* subtype. Occasionally mixed subtypes have been reported in *adwr, adyw, adywr* and *aywr*. Both apparently excluded determinants map to the same molecule. It is not known if this represents a double infection or an uncommon HBV strain.

Virion DNA: It has been mentioned above, that the HBV genome is a small circular partially double-stranded DNA molecule. This genome is accompanied by a single stranded region of variable length (Fig. 1). The minus (−) strand is linear and of fixed length of about 3200 nucleotides. It is the coding strand from which the viral mRNA and the viral pregenomic RNA are transcribed. At its 5′ end, there is a protein that serves as primer for reverse transcription. The plus strand (+) is of variable length ranging from 50 to 100 % that of the minus strand. The maintenance of the circular structure is assured by a short cohesive overlap region of about 200 nucleotides at the 5′ end of the two strands. A 12 bp direct repeats (DRI and DRII) located near the 5′ end of both strands seems to serve as a primary site for replication (Fig. 1).

The first «T» of the sequence 5′GAATTC in the plus strand corresponding to the unique *EcoRI* site which exists in most genomes is used as reference origin of physical map. Nucleotides numbering is from 5′ to 3′ in the plus strand. When the *EcoRI* site dose not exist the base occupying the same position is taken as position 1.

Physical structure and genetic organization of the HBV genome: As a remind and completion, it is pointing out that the hepatitis B virus genome contains four overlapping open reading frames genes encoding all known proteins of this virus. The envelope open reading frame, or S region (S gene), contains three in phase translation start codons (ATG) defining the N-termini of three envelope polypeptides; pre-S1, pre-S2 and S protein (The hepatitis B surface antigen [HBsAg]) [54, 81, 82]. Referring to the *ayw* subtype [47], the shortest polypeptide (226 aa) (aa = amino acid) that contains the group *a* and the subtypes (*d/y, w/r*) determinants is also called major (small) protein (HBsAg) because of in relative abundance. It is encoded by the S region starting from the ATG at position 158 (in the *adw* subtype [83]). The middle envelope polypeptide contains the entire amino acid sequence of the major polypeptide plus 55 aa at the N-terminus containing the pre-S2 antigen, starting from the ATG at position 3214 ([The P31 ATG] in *adw* subtype [83]). The large protein is composed by the entire amino acid sequence of the middle envelope polypeptide plus 158 aa at the N-terminus and bears the pre-S1 antigen [52, 82]. The two pre-S antigens are highly immunogenic neutralizing epitopes, the larger one being involved in the virus binding to cell receptors and in the entry in the hepatocytes [82]. Many transcriptional factors are able to bind to specific sites in

the pre-S region of HBV DNA and could be somehow responsible for the hepatotropism i. e. HNF1 and API [83, 84].

A glucocorticoid responsive element (GRE) has been mapped within the S gene between positions 351 and 366 (in *ayw* subtype) [85]. This element has no enhancer activity but act synergettically with the viral enhancer [86]. Steroid hormones have been shown to positively regulate the S gene expression in transgenic mice [87]. (The Enhancer activity will discussed in other part of this review.) The capside open reading frame or region C (C gene) contains two in frame ATG and encodes a nucleic acid binding protein, HBcAg, that encapsidate the viral nucleic acids and a 29 aa longer polypeptide (pre-C) that is secreted as HBeAg [88]. The X region (X gene) encodes a polypeptide expressed during HBV infection and in hepatocellular carcinoma [89]. This polypeptide has transactivating properties on HBV and other viral and cellular promoters [90—92]. The P region (P = Pol gene) overlaps all the others. It codes for a protein with several function in replication; (DNA polymerase, RNAse H, primer for DNA synthesis and reverse transcriptase [93]). This protein as well as reverse transcriptase activity [94, 95], binds to the viral DNA 5′ terminus of the minus strand [96].

Three major classes of HBV-specific message are detected in infected hepatocytes, and 5′ ends of the RNAs are heterogeneous (Fig. 2) [41, 54, 97]: the 3.5-kb RNAs, which are slightly larger than the 3.2 kb unit length of HBV genome serve as the mRNAs for expression of the core protein. It is also used for the synthesis of viral DNA polymerase [7, 8, 98]. Because these RNAs are the only species containing the full complement of viral genetic information, they also serve as templates for reverse transcription during HBV replication [8, 97, 99]. Two other mRNAs are subgenomic in size: the 2.4 kb RNAs encode the large envelope protein HBV surface antigen (S-protein), and the 2.1 kb RNAs encode the middle and major HBV surface antigens. Expression of these HBV-specific mRNAs is controlled by four different promoters in the HBV genome [54]: the Core promoter regulates expression of the 3.5 kb RNAs, whereas the pre-S promoter (The distal TATA-like promoter (SPI)) [4, 100, 101] and the S promoter (The proximal Simian virus 40 (SV40)-like promoter (SPII)) regulate expression of the 2.4 and 2.1 RNAs, respectively [44, 102, 103]. Both the SPI and the SPII promoters display a preference for differentiated hepatoma cell lines [104]. The liver- and differentiated state-specific transcriptional activities of the SPI promoter are controlled by the combined action of a HNF-1 binding element, lying between 68 and 95 bp upstream of the RNA cap site in the SPI promoter region [98], and the HBV enhancer, which is located downstream of the coding sequence of the S gene [104, 105].

The liver- and differentiated state-specific transcriptional activities of the SPII promoter are contributed mainly by the upstream flanking sequence in the promoter region [83, 104, 106]. In addition (0.7

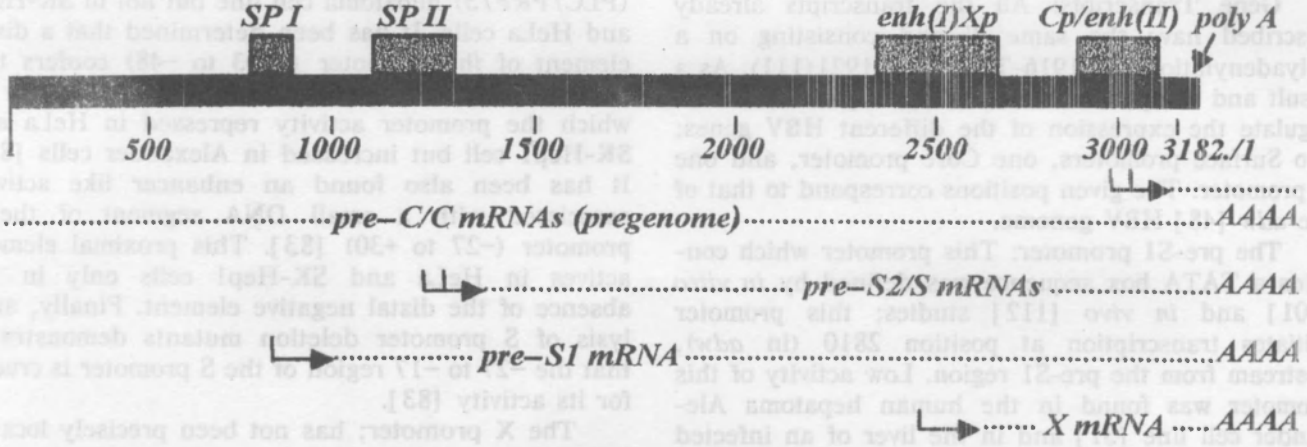

Fig. 2. Linear representation of the HBV genome. The numbering system of Pasek et al. [48] is used. Transcriptional control regions are shown as boxes and are abbreviated as follows: *SPI* = pre-SI promoter; *SPII* = pre-S2/S promoter, *enh(I)/Xp* = enhancer X/I promoter, *Cp/enh(II)* = Core promoter/enhancer II

[113]) 0.8 to 0.9 kb mRNA has been detected in the *in vitro* expression system by the DNA transfection of HBV DNA [107]. This transcript may be related in some way to the expression of the X gene [99], controlled by X-promoter. One of the two major HBV-specific poly(A+) RNAs characterized in infected livers of chimpanzees is 2.1 kb long [4, 41]. This transcript codes for the major S protein and apparently for pre-S2 protein. Thus, four classes of mRNAs have been identified so far in the process of HBV propagation and are known to use the single polyadenylation signal for their termination [99].

Two similar major transcripts were also observed in the infection of other hepadnaviruses, the woodchuck hepatitis virus (WHV) and ground squirrel hepatitis virus (GSHV). Analysis of the 5′ ends of the two major transcripts from infected woodchucks and ground squirrels indicated that they were heterogeneous for all transcripts [108, 109]. For the two major transcripts of HBV, however, use of the transient expression system of transfected HBV DNA with HuH-7 cell made possible a similar analysis of the heterogeneity of 5′ ends of two major transcripts [67]. It is mentioned above, that in human, the principal site of clinical pathology after HBV infections is the liver because HBV actively replicates only in hepatocytes [17, 41]. Consistent with this observation, the 3.5 kb genomic transcript has been detected primarily in well-differentiated human hepatoma cell lines transfected by the cloned HBV genome [67, 71], suggesting that liver-specific factors are needed for efficient transcription of the genomic transcripts from the core promoter, but although HBV DNA has been found in non-hepatic tissues in infected patients and in transgenic mice [38, 39, 42, 43, 110].

Gene Transcripts: All the transcripts already described have the same 3′ end consisting on a polyadenylation site 1916-TATAAA- 1921(111). As a result and completion, there are four promoters that regulate the expression of the different HBV genes; two Surface promoters, one Core promoter, and one X promoter. The given positions correspond to that of the *adw* [48] HBV genome.

The pre-S1 promoter: This promoter which contains a TATA box sequence was defined by *in vitro* [101] and *in vivo* [112] studies; this promoter initiates transcription at position 2810 (in *adw*), upstream from the pre-S1 region. Low activity of this promoter was found in the human hepatoma Alexander cell line [31] and in the liver of an infected chimpanzee [4]. Using stable transformation with cloned HBV DNA, it was shown that this TATA box-containing promoter is not essential for the expression of hepatitis B surface antigen [76]. Thus the pre-S1 promoter, which is a canonical TATA sequence, located upstream the ATG of the pre-S1 region and, produces 2.4 kb transcripts. However, this promoter is less functional that the other viral promoters [82].

The S promoter; as a second promoter termed the S promoter and was characterized and mapped at the pre-S2 region. This promoter induces initiation of transcription at three major sites, spanning some 30 bp) around the *EcoRI* site [4, 5, 76]. The position of these three initiation sites are 5 nucleotides downstream from and 5 and 25 nucleotides upstream from the *EcoRI* site (designated as *a*, *b*, and *c*, respectively). Since the ATG of the pre-S2 region is positioned between the *b* and *c* initiation sites, only the two longest RNA species may code for p31 protein with an extra 55 amino acids of the pre-S2 region. The shorter species lack the p31 ATG so that the first available ATG, at position 158 (in *adw* subtype), is the transcriptional start point of the major S protein. It was proposed that the expression of these transcripts *in vivo* is directed by a sequence positioned around the *Fnu4HI* restriction site (3165 in *adw* subtype) [4] at the region where sequence similarity to the SV40 late promoter was been shown. Thus the S promoter is located round position 3155 (in *ayw* subtype) just upstream to the translation site of the middle protein and produces 2.1 kb transcripts [4]. The 5′ ends of these transcripts are heterogeneous and encodes both the major (small) and middle protein. Despite the extensive mapping of S gene mRNA initiation sites, little is known about regulatory elements which modulate the S promoter.

Transient expression studies revealed that this promoter is highly active in the Alexander (PLC/PRF/5) hepatoma cell line but not in SK-Hepl and HeLa cells. It has been determined that a distal element of the promoter (−103 to −48) confers this cell-type-specific behavior through a mechanism in which the promoter activity repressed in HeLa and SK-Hepl cell but increased in Alexander cells [83]. It has been also found an enhancer like activity associated with a small DNA segment of the S promoter (−27 to +30) [83]. This proximal element actives in HeLa and SK-Hepl cells only in the absence of the distal negative element. Finally, analysis of S promoter deletion mutants demonstrated that the −27 to −17 region of the S promoter is crucial for its activity [83].

The X promoter; has not been precisely located [107]. It is weakly active *in vivo* and represent less than 1 % of the viral transcripts. In *in vitro* system, 0.7 kb and 0.9 kb transcripts have been reported [113]. This promoter seem to be more efficient out of

the whole HBV genome context. Since the HBV X protein (pX) *trans*-activates many promoters [114], including the HBV Core promoter [91], it is determined that pX not regulates the pre-S1 or S promoter (pX has not *trans*-effect on the Surface gene promoters).

The Core promoter; regulates the replication of the virus, as the 3.5 kb C mRNA/pregenome not only serves for translation of the Core and Pol proteins but also represents the template for reverse transcription [97, 115, 116]. From the second Core promoter transcript, the pre-C mRNA, only the HBeAg precursor is translated [115, 116]. The Core promoter is composed of a minimal or basic Core promoter (BCP) sufficient to initiate transcription and of upstream regulatory sequences (URS) [117—119]. A short TA-rich sequence in the BCP serves as both the initiator and TATA box for transcription initiation of the pre-C mRNA and C mRNA/pregenome, respectively [120].

An important activating URS element is the alpha box (see part of Regulatory Elements of HBV genome in this review), which binds hepatocyte nuclear factor 4 (HNF4), C/EBP, or other liver-specific transcription factors [110, 117, 118, 121—124]. In addition, binding sites for HNF3 and ubiquitous factors like SPI were identified in this promoter [125, 126]. Unlike the pre-S1 promoter, the Core promoter of wild-type HBV does not contain an HNF1 binding site [127].

Because of its crucial role in the viral life cycle, naturally occurring sequence variation in the Core promoter of HBV in patients is under intense investigation. Specific point mutations in the BCP were found in HBV from patients with fulminant hepatitis [128—131]. Similar mutation as well as different short deletions or insertions were found in viremic HBeAg-negative patients with chronic hepatitis B [129—134].

Since for these patients no mutation in the C gene could explain the lack of HBeAg expression, it was speculated that promoter mutation may be responsible. Specific types of short deletions in the Core promoter region were found in patients with extremely low-level viremia, in some cases without any serological marker for HBV infection [135—138]. Thus, a particular phenotype may be caused by specific mutations in the Core promoter. However, this speculation has not previously been substantiated by experimental evidence [93]. At last the Core promoter, produces transcripts of 3.5 kb. Three 5′ ends have been located upstream of the C gene: two of them initiating downstream of the ATG of pre-C region, coding for the major capsid antigen (HBcAg) and the genomic viral DNA, and another one starting upstream region pre-C coding for the HBeAg [67]. A C gene specific 2.1 kb spliced transcript that represent the 2.1 kb S transcripts has been described [139].

A polymerase gene promoter has not been identified. The 3.5 kb transcripts that have heterogeneous 5′ ends [108] could also encode the polymerase gene products.

Regulatory Elements of HBV genome: Eukaryotic gene expression is in large part regulated at the transcription level. Such regulation is governed by the constellation of *trans*-acting cellular factors that bind to specific *cis*-acting elements and act in either a positive or negative manner [140]. Cell-type-specific gene activation a primary determinant of cellular differentiation, represents a more complex type of interplay, as both constitutive and tissue-restricted *trans*-acting regulatory factors are involved [141]. The differential sequence-specific recognition of these *cis*-acting elements in promoters and/or enhancers by their cognate factors provides a mechanistic basis for the tissue- and differentiation-specific regulation of gene expression. The study of model viral genes which display distinct tissue tropism can provide valuable insight into the intricate effects of cell-type-specific transcription regulation on differentiation. It has been mentioned above, that control of gene transcription in part regulated by the presence of *cis*-acting DNA elements that interact with specific nuclear transcription factors. Enhancers, which act in a position- and orientation-independent mannner, are key regulatory elements in the transcriptional regulation of viral and cellular genes [142, 143]. The 72-bp repeat of simian virus 40 (SV40) is the best-characterized enhancer.

The SV40 72-bp enhancer, which is composed of multiple regulatory *cis*-acting elements via the interaction whit ubiquitous and cell-type-specific transcriptional factors, orchestrates the expression of viral genes in many cells [144—146]. Two regions of the HBV genome are known to display properties of a transcriptional enhancer (Fig. 2) [147—149]. A transcriptional region, Enhancer I, is located between the open reading frames of the surface antigen, within the region P, and upstream the X region (position 1074—1234 in *ayw* subtype) and partially overlaps the X promoter [147, 150]. Because activation of transcription by this enhancer is greater in several cultured hepatoma cells than in nonhepatic cells, it has been suggested that this enhancer is responsible for liver-specific gene expression of HBV [150]. It is described the identification of a second enhancer sequence (enhancer II) in the HBV genome: Enhancer II is situated downstream of the previously identified en-

hancer (enhancer I), immediately upstream from the coding region of the Core gene (initiation site of viral major transcript) [152], overlaps with the Core promoter [93], within the X open reading frame [98, 152].

Enhancer II has been mapped to nt 1636 to 1741 [123] in HBV genome. It furnishes a unique model of use in investigating the structure and function of an enhancer. Unlike enhancer I, the activity of enhancer II is highly liver specific, functioning only in highly differentiated human hepatoma cells. Furthermore, enhancer II activity varies in different hepatoma lines, suggesting that this enhancer is regulated according to the differentiation state of the hepatoma line used. Because enhancers have been shown to play a pivotal role in the regulation of mammalian and viral gene expression and because HBV gene expression is tightly coupled to the step of reveres transcription in this replication cycle, a mechanism for regulation of HBV enhancer activity may clarify the molecular basis for the absence of HBV replication and gene expression in hepatocellular carcinoma [22]. With various deletions at the 5′ end of enhancer II, a positive regulatory element was identified at nt 1636 to 1690 (the II-A element), with the 5′ boundary between nt 1636 and 1671. The II-A Element alone did not have an enhancer function, but the enhancer activity was been achieved by the concomitant presence of the sequence from nt 1704 to 1741 (the II-B element). The II-B Element alone did not have enhancer activity. These facts indicate that cooperation between the II-A and II-B elements is required to exhibit the enhancer activity of enhancer II [98]. Two functional constituents, a 23-bp sequence box-α in the II-A element and a 12-bp sequence box-β in II-B element, were identified as being both necessary and sufficient for enhancer II function [123]. Interestingly, either box-α or box-β in an upstream position can regulate the activity of the nearly Core promoter [119].

Examination of the box-α and box-β sequences reveals a weak homology to the extended consensus for a C/EBP binding site. Gel shift and footprinting analyses indicate that multiple proteins bind to these sequences and thus are candidate transcription factors that mediate the enhancer function. One heat-resistant protein, protein *a*, and one heat-sensitive protein, protein *b*, bind to box-α. Protein *a*, which bind to box-α in a way indistinguishable from that seen with a recombinant C/EBP, appears not to be identical to C/EBP in that the binding of protein *a* requires a minimal sequence larger than the canonical C/EBP sites.

Two box-β-binding proteins *c* and *d*, show greater affinity for the C/EBP consensus than for box-β. However, both proteins *c* and *d* are relatively heat sensitive and display a distinct sequence preference from the recombinant C/EBP protein. Since the function of enhancer II is strictly dependent on a bipartite architecture, this system provides a unique model for studies of how the interactions of its binding proteins lead to the enhancer function. Furthermore, proteins that display binding activities to box-α and box-β are found to be present in nuclear extracts of the differentiated human hepatoma cell line HepG2 by DNAse I footprinting and gel shift analyses [123]. Using DNA transfection to bypass viral entry into cells, it has been demonstrated that the expression of HBV genes exhibits liver cell and differentiation state specificity in the infective process *in vivo* [64, 66, 67, 71, 104, 153—155]. Previous studies show that only in the human hepatoma cell lines HepG2 and HuH-7, which have the feature of well-differentiated liver cells, does enhancer II have strong enhancing activity on the simian virus 40 (SV40) early promoter [110]. In contrast, this is not seen in the poorly differentiated HA22T/VGH cells or the nonliver HeLa cells [98]. These results also apply to the upstream regulatory effect on the basal Core promoter (BCP) [119]. These differentiated actions, therefore, may contribute at least in part to the observed hepatotropism of HBV. It has been mentioned above that gel shift experiments reveal a unique box-α-binding protein, protein *a*, which is present only in differentiated liver cells. where enhancer II is functional. The converse is true for another box-α-binding protein, protein *f*, which is present only in poorly differentiated liver cells and nonliver cells. The simplest hypothesis that explains these results is that protein *a* activates and/or protein *f* suppresses the enhancer and upstream regulator functions.

Although C/EBP is a candidate for a transcription factor that interacts with box-α or box-β, none of the binding factors identified in the gel shift assays, including protein *a* and protein *f*, is likely to be C/EBP because they differ from C/EBP in heat liability and sequence preference [117]. In addition, enhancer II consists an upstream negative regulatory element [98, 110, 122, 124, 152, 156]. Enhancer II activates the transcriptional activity of both the SPI and SPII promoters in a liver- and differentiated state-specific manner [98].

It has been shown that the two HBV enhancers strongly effect the activity of all three major HBV promoters in human hepatoma cells and that the activity of HBV enhancers is differentially regulated, depending on the state of hepatocyte differentiation.

Ф. Аджаміян

Вірус гепатиту В: загальний опис, фізична структура, генетична організація, транскрипція генів та геномні регулюючі елементи

Резюме

Геном вірусу гепатиту В людини (HBV) існує у вигляді дволанцюгових кільцевих молекул ДНК. Він містить чотири фланкуючі відкриті рамки зчитування (ORF_S) генів. S (preS1, preS2) регіон(и) кодує головний, середній та великий білки (HBsAg). С і preC регіони кодують HBcAg і HBeAg. X регіон кодує поліпептид, який експресується за час HBV інфекції. P регіон кодує білок з різноманітними функціями у реплікації. Ідентифіковано чотири класи HBV мРНК. У геномі HBV pre-S1 промотор продукує великий білок, pre-S2/S виробляє головний і середній білки. X промотор кодує 0,7 і 0,9 транскрипти. С і пре-С промотори кодують Core і pol(HBVcAg) і HBeAg білки. Ключовими регуляторними елементами HBV є енхансери I і II. Активність енхансера II є специфічною для печінки. Він активує транскрипцію обох пре-S1 і пре-S2/S промоторів. Два HBV енхансери активують основні промотори HBV. Бокс-α в II-A і бокс-β в II-B елементах у геномі HBV необхідні для функції енхансера II. Бокси α і β можуть регулювати активацію промотора Core, білки a, b, f і білки c, d прикріплюються у боксах α і β відповідно і впливають на енхансерну функцію.

Ф. Аджамиян

Вирус гепатита В: общее описание, физическая структура, генетическая организация, транскрипция генов и геномные регулирующие элементы

Резюме

Геном вируса гепатита В человека (HBV) существует в виде двухцепочных кольцевых молекуул ДНК. Он содержит четыре фланкирующие открытые рамки считывания (ORF_s) генов. S (preS1, preS2) регион(ы) кодирует главный, средний и большой белки (HBsAg). С и пре-С регионы кодируют HBcAg и HBeAg. X регион кодирует полипептид, экспрессирующийся в течение HBV инфекции. P регион кодирует белок с разнообразными функциями в репликации. Идентифицированы четыре класса HBV мРНК. В геноме HBV пре-S1 промотор продуцирует большой белок, пре-S2/S промотор производит оба (главный и средний) белка. X промотор кодирует 0,7 и 0,9 транскрипты. С и пре-С промоторы кодируют Core и pol(HBcAg) и HBeAg белки. Ключевыми регуляторными элементами в HBV являются энхансеры I и II. Активность энхансера II очень специфична для печени. Энхансер II активирует транскрипционную активность пре-S1 и пре-S2/S промоторов. Два HBV энхансера активируют основные промоторы HBV. Бокс-α в II-A и бокс-β в II-B элементах в геноме HBV необходимы для функции энхансера II. Боксы α и β могут регулировать активацию Core промотора, белки a, b, f и белки c, d прикрепляются в боксах α и β соответственно и действуют на энхансерную функцию.

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