JOURNAL OF VIRAL HEPATITIS
JVH

Journal of Viral Hepatitis, 2010, **17**, 527–536 doi:10.1111/j.1365-2893.2010.01315.x

REVIEW

Control of hepatitis B virus at the level of transcription

M. Quasdorff[1] and U. Protzer[2] [1]*Department of Gastroenterology and Hepatology, University Hospital Cologne, Germany; and* [2]*Institute of Virology, Technische Universität München/Helmholtz Zentrum München, Munich, Germany*

Received February 2010; accepted for publication March 2010

SUMMARY. Hepatitis B virus (HBV) is tightly controlled by a number of noncytotoxic mechanisms. This control occurs within the host hepatocyte at different steps of the HBV replication cycle. HBV persists by establishing a nuclear minichromosome, HBV cccDNA, serving as a transcription template for the viral pregenome and viral mRNAs. Nucleoside/nucleotide analogues widely used for antiviral therapy as well as most antiviral cytokines act at steps after transcription of HBV RNAs and thus can control virus replication but do not directly affect its gene expression. Control of HBV at the level of transcription in contrast is able to restrict both, HBV replication and gene expression. In the review, we focus on how HBV is controlled at the level of transcription. We discuss how the composition of transcription factors determines HBV gene expression and replication and how this may be influenced by antivirally active substances, e.g. the cytokine IL-6 or helioxanthin analogues, or by the differentiation state of the hepatocyte.

Keywords: antivirals, HBV, hepatocyte differentiation, nuclear receptor, transcription factor.

THE HUMAN HEPATITIS B VIRUS (HBV)

The human HBV is the prototype member of the family of *hepadnaviruses* (stands for *hepatotropic DNA viruses*). These are small, enveloped DNA viruses characterized by a pronounced liver tropism and replication via reverse transcription of an RNA pregenome [1].

Hepatitis B virus is a noncytopathic virus because its assembly in hepatocytes proceeds without cell disruption. This has been demonstrated in stably transfected hepatoma cell lines [2] as well as in HBV-transgenic mice [3] and explains why HBV infection *per se* does not cause liver damage and elicits little innate immune response [4]. Liver damage is rather a consequence of the activation of adaptive immune responses. Because HBV-producing cells are long lived and continuously release progeny viral particles, HBV replication is tightly regulated within hepatocytes.

The HBV genome (3.2 kb) consists of a partially double-stranded, relaxed circular (rc) DNA and is extremely compactly organized with widely overlapping open reading frames encoding the four structural and two nonstructural viral proteins. In addition, regulatory elements like promoters, enhancers and the polyadenylation and encapsidation signals overlap with coding sequences [5] (Fig. 1).

Hepatitis B virus enters hepatocytes by a so far unknown mechanism. Following fusion of viral and cellular membranes, the viral capsid is transported to the nuclear pore complex where the rc DNA genome is released into the hepatocyte nucleus. Inside the nucleus, rcDNA is converted to a covalently closed circular DNA (ccc DNA) by cellular enzymes. cccDNA serves as template for transcription of the 3.5-kb pregenomic/precore (pre-C) RNAs from the pre-C/pregenomic promoter, which both together are named core or nucleocapsid promoter. Subgenomic RNAs of 2.4 kb and 2.1 kb initiate from preS1 and preS2/S promoters, respectively, and a 0.7-kb RNA from the X promoter (Fig. 1). Two enhancer (Enh) elements were indentified within the HBV genome: Enh I and Enh II. Enh I is a 270-nt region located between S and X open reading frames, which consists of a 5′ modulatory element, a central core domain functioning as the actual enhancer element and a 3′ domain. Enh II is a 105-bp region located upstream of the basal part of the core promoter and partially overlaps with its core upstream regulatory sequences. Figure 2 gives an overview about HBV

Abbreviations: AP-1, activator protein 1; C/EBP, CAAT enhancer-binding protein; COUP-TF, chicken ovalbumin upstream promoter transcription factor; CREB, C-AMP-response element binding protein; ED, embryonic day; FTF, fetoprotein transcription factor; FXR, farnesoid X receptor; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HNF, hepatocyte nuclear factor; NF, nuclear factor; NRF1, nuclear respiratory factor 1; Oct1, octamer transcription factor 1; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator 1-α; PPAR, peroxisome proliferator-activated receptors; RXR, retinoic X receptor; SHP, small heterodimer partner; TBP, TATA binding protein; TR4, testicular orphan receptor 4.

Correspondence: Prof Ulrike Protzer, MD, Institute of Virology, Technische Universität München/Helmholtz Zentrum München, Trogerstr. 30, D-81675 München, Germany. E-mail: protzer@tum.de; protzer@helmholtz-muenchen.de

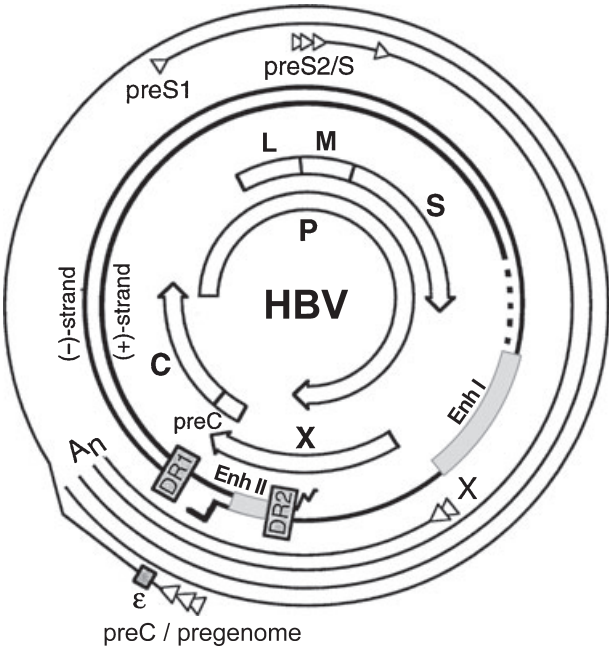

Fig. 1 Genome organization of hepatitis B virus (HBV). The HBV genome is a 3.2 kb, partially double stranded, relaxed circular DNA represented by the bold inner circles. The thin, outer lines represent the different classes of transcripts (3.5, 2.4, 2.1 and 0.7 kb) with arrowheads marking the start sites. Open arrows in the centre represent the four open reading frames encoding for the viral proteins (C, core protein; preC/C, hepatitis B e antigen; envelope proteins L, M and S; P, polymerase; X, X protein). Enhancer (Enh) I und II, direct repeat (DR) 1 and 2, the common polyadenylation site (An) and the encapsidation signal ϵ are indicated.

promoter/enhancer elements and the transcriptional regulators reported to bind, which are detailed in the following sections.

The pregenomic RNA serves as mRNA for core and polymerase/reverse transcriptase proteins. The pre-C RNA initiates only a few nucleotides upstream as mRNA for the secreted hepatitis B e antigen (HBeAg). These RNA species contain a hairpin-loop at the 5′-end, which trigger encapsidation together with the viral polymerase/reverse transcription into an icosahedral capsid. The capsid in the virion consists of 240 subunits of the HBV core protein, which organize to some extent the surrounding envelope proteins [6]. The viral envelope is built of a lipid bilayer and is densely packed with the large (L), middle (M) and – predominantly – the small (S) envelope proteins. L, M and S proteins share the S domain, L and M proteins contain additional preS1 and preS1 domains at the N-terminus. A third subgenomic RNA encodes the regulatory protein X, which is required to establish infection *in vivo* [7,8].

UBIQUITOUS TRANSCRIPTION FACTORS AND TRANSCRIPTION OF HBV GENES

Efficient transcription of HBV genes requires a number of ubiquitous transcription factors. The *nuclear factor-1 (NF-1)* family of transcription factors participates in transcriptional regulation of a great number of genes in many different cell types [9]. Three NF-1 binding sites with apparently different functions were identified within the HBV genome. Shaul *et al.* [10] showed that NF-1 is essential for optimal activity of the S-promoter binding approximately 190 bases upstream. Spandau *et al.* [11] defined another NF 1-binding site between HBV Enh I and core promoter that was responsible for the suppression of the HBV enhancer

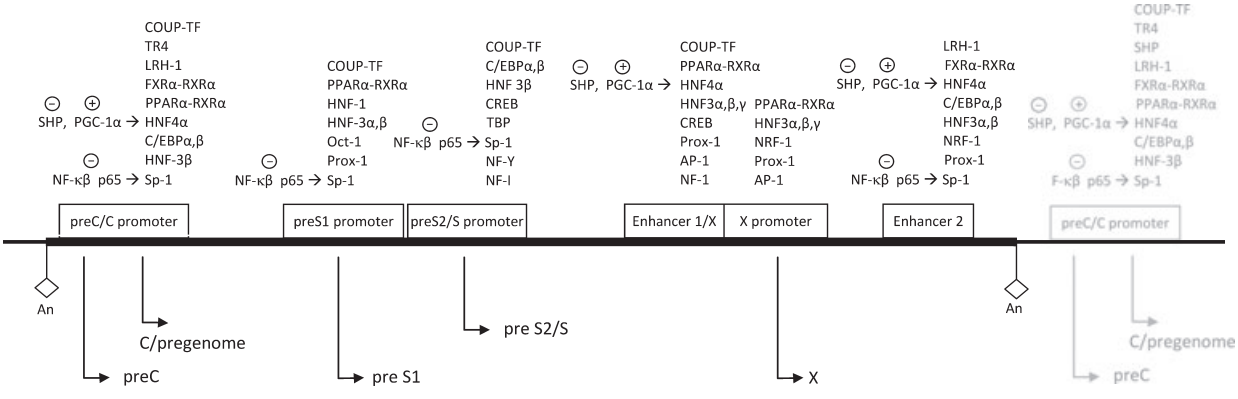

Fig. 2 Binding sites of ubiquitous and hepatocyte-enriched transcription factors within HBV promoter and enhancer regions. The different HBV promoter and enhancer sites are schematically depicted as boxes. To facilitate allocation, the HBV genome is linearized, and the parts used twice during transcription of 3.5 kb RNAs are depicted in 5′ and in 3′. Angled arrows indicate HBV RNA start sites, open diamonds indicate the polyadenylation site (An). Transcription factors and nuclear receptors binding are listed above the respective promoter. Arrows indicate an indirect influence of a transcription factor via another transcription factor. A positive indirect influence is hallmarked with a plus (+) and a negative with a minus sign (−).

function. The third NF-1 binding site within the HBV Enh I was identified by Ori *et al.* [12], who demonstrated its central role in the regulation of HBV gene transcription because mutation almost abolished the synthesis of the HBV 3.5-kb RNA. Another member of NF-1 family, NF-Y, was found to bind to CCAAT element of the S promoter and up-regulate it.

Specificity protein 1 (Sp1) is an ubiquitous transcription factor that binds to guanine-cytosine-rich DNA elements and is involved in gene expression during early development of an organism [13]. Several Sp1-binding sites were identified on the HBV genome. Exogenously expressed Sp1 binds and activates the preS2/S promoter [14], the preS1 promoter [15] and core promoter and Enh II [16]. Li *et al.* [17] determined that Sp1-binding site in the Enh II positively regulated transcription of all HBV genes, those in the core promoter only transcription of HBV core and pre-C RNAs. The upstream Sp1-binding site in contrast was described to negatively regulate transcription of S and X genes. Thus, NF-1 and Sp1 probably contribute to differential regulation of HBV gene expression during natural viral infection.

The *activator protein 1 (AP-1)* is a heterodimeric transcription factor composed of proteins belonging to the c-Fos, c-Jun, ATF and JDP families [18]. AP-1 was shown to be involved in the up-regulation of the HBx protein expression by insulin [19] and by HPV-16 E6 [20].

TATA binding protein (TBP) is a transcription factor that specifically binds to the TATA box DNA sequence [21]. Bogomolski-Yahalom *et al.* [22] showed that TBP binds to the putative TATA-like sequences and the initiator sequence in the pre-S2/S promoter region that is necessary for the promoter activity.

Prospero-related homeobox protein (Prox1) is a homeobox-containing transcription factor that represses expression of a number of genes by interacting with LRH-1 [23]. Using reporter-gene analysis, Qin J *et al.* [24] showed that Enh II/core, preS1 and Enh I/X promoter are targets for Prox1-mediated repression. Detailed analysis revealed that Prox1 down-regulates LRH-1-mediated activation of Enh II/core promoter, whereas it represses preS1 activity by interacting directly with hepatocyte nuclear factor (HNF) 1.

C-AMP-response element binding protein (CREB) transcription factors are involved in gluconeogenesis, lipid metabolism and hepatocyte proliferation [25,26]. The binding of CREBs seems to be enhanced in inflammation [27]. Recently, Tacke *et al.* [28] showed that the CREB activator protein kinase A increased 2.1-kb RNA transcription and S-protein expression, whereas levels of pregenomic/pre-C RNA were not affected. *Kim et al.* [29] showed the requirement of CREB for expression of all HBV antigens, HBV pregenomic RNA and virus replication. CREB binds to a motif in Enh I that is highly conserved among published HBV sequences. Thus, CREB also contributes to adapt HBV gene transcription to different metabolic stimuli and hepatic inflammation.

The *nuclear factor-kappa B (NF-κB)* transcription factor family members p50 and p65 are critical regulators of the immediate early pathogen response and play an important role in promoting inflammation, regulation of cell proliferation, differentiation, survival and tumorigenesis. NF-κB transcription factors can form homo- or heterodimers, but the p50-p65 heterodimer is the major NF-κB dimer in many cells including hepatocytes [30]. Lin *et al.* demonstrated that NF-κB p65 indirectly inhibits synthesis of all HBV RNAs. p65 binds Sp1 protein at its N-terminus repressing the binding of Sp1 to Sp1 sites in HBV genome and thus inhibiting HBV gene expression [31].

Octamer transcription factor 1 (Oct1) binds to the preS1 promoter near the HNF1-binding site. Although Oct-1 alone was not able to activate transcription from the preS1 promoter, it seems to be an essential co-activator of HNF1.

Nuclear respiratory factor 1 (NRF1) activates the majority of nucleus-encoded mitochondrial genes and various housekeeping genes. Tokusumi *et al.* [32] showed binding of NRF1 to a minimal X promoter and positive regulation of X gene transcription.

Although ubiquitous transcription factors do not confer hepatocyte tropism to HBV, they are essential for basal activity of HBV promoters and enhancers, for differential expression of HBV genes as well as for adaptation of HBV gene transcription to changes in the intra- and extracellular milieu.

LIVER-ENRICHED TRANSCRIPTION FACTORS

A number of liver-enriched transcription factors and nuclear receptors have been shown to bind HBV promotor/enhancer elements and to be critical in activating and regulating HBV transcription. In addition, they determine – besides the so far unknown virus receptor – the hepatocyte tropism of HBV.

Hepatocyte nuclear factor 3

Hepatocyte nuclear factor 3α (Foxa1), HNF3β (Foxa2) and HNF3γ (Foxa3) bind to similar DNA target sequences within hepatocyte-specific regulatory regions and show functional redundancy in hepatocytes. They mainly regulate carbohydrate metabolism and β-oxidation of lipids [33]. Chen *et al.* [34] found that HNF3α, HNF3β and HNF3γ bind to HBV Enh I and increase its activity 15-fold. Li *et al.* [35] showed that HNF3α and HNF3β bind to HBV Enh II and activate it in a dose-dependent manner. Moreover, in HeLa cells, HNF3β switched on Enh II which normally is not active in these cells. In HepG2.1 cells, HNF3α and HNF3β increased transcriptional activity of the preS1 promoter 29-fold and 86-fold, respectively [36]. In addition, HNF3 was found to bind to the preS2/S promoter [37]. While HNF3α did not affect the level of transcription from the other HBV promoters, HNF3β even decreased synthesis of the HBV pregenomic and pre-C RNAs by interfering with RNA elongation [38]. Because HNF3 was shown to be able to bind its sites in compacted chromatin and to open the local nucleosomal domain in the absence of adenosintriphosphate-dependent

enzymes [39], it is reasonable to speculate that the key role of HNF3 in the context of HBV may be to bind to the HBV cccDNA minichromosome and thus to provide access for other transcriptional regulators.

CAAT enhancer-binding protein (C/EBP)

CAAT enhancer-binding protein α and β proteins are co-expressed in hepatocytes and are able to form either homo- or heterodimers for sequence-specific DNA binding [33]. C/EBP is involved in the regulation of hepatocyte glucose and lipid homeostasis and hepatocyte proliferation [40]. Lopez-Cabrera *et al.* [41] showed that C/EBP can bind and activate the HBV Enh II in a dose-dependent manner. In addition, C/EBP binds to the core promoter and modestly activates transcription at low but represses it at high concentrations [42]. Another binding site of C/EBP was found in the S promoter and shown to be necessary for full S-promoter activity [2]. Choi *et al.* [43] proposed an interaction between the basic leucine zipper domain of C/EBPα and HBx protein enhancing transcription from the HBV pregenomic promoter.

Hepatocyte nuclear factor 1

Hepatocyte nuclear factor 1 is expressed in two isoforms: HNF1α and HNF1β (vHNF1). In the liver, HNF1α forms heterodimers with HNF1β-related family member [40]. HNF1α binds 6% of RNA polymerase II-enriched promoters in hepatocytes [44], is an important regulator of glucose and amino acid homeostasis [45] and contributes to liver development, maintenance of differentiated and polarized hepatocytes and normal liver structure [46]. Raney *et al.* [47] found that HNF1α increases transcription from the preS1 promoter approximately 10-fold in HepG2.1 and 7-fold in HuH7 cells, whereas it did not activate the other three HBV promoters. Activation of the preS1 promoter depends on interaction with ubiquitous transcription factor Oct1 [48]. Thus, HNF1α was found to exclusively regulate the expression of 2.4-kb preS1 transcript. By modulating expression of the envelope protein L, HNF1α can regulate release of virions. The confusing observation that transcription was not affected in HNF1α -deficient, HBV-transgenic mice [49] might be attributed to a compensation by other liver-enriched transcription factors.

NUCLEAR RECEPTORS IN HEPATOCYTES

Hepatocyte nuclear factor 4

HNF4α is an orphan nuclear receptor that forms homo- or heterodimers with retinoic X receptor α (RXRα). Forty-two per cent of all hepatocyte genes are regulated by HNF4α [44]. HNF4α is the key regulator of carbohydrate, lipid, cholesterol, amino and bile acid homeostasis and is essential for formation of epithelial phenotype of hepatocytes, hepatocyte differentiation and normal liver structure [40].

Raney *et al.* [37] overexpressed HNF4α in HepG2.1 cells and found that it increased the level of transcription from preS1, preS2/S and core promoters approximately nine-, three,- and twofold, respectively, whereas it did not affect the Enh I/X promoter. They identified two HNF4α-binding sites in the core promoter and one in the Enh I/X promoter region. Garcia *et al.* [50] found that HNF4α binding to Enh I transactivated the core promoter. Later studies by Yu and Mertz [51] identified genetically distinct pre-C and pregenomic RNA promoters within the core promoter region each consisting of its own transcriptional initiator and a TATA box-like sequence. HNF4α inhibited transcription from the pre-C promoter and activated transcription from the pregenomic promoter [52]. Overexpression of HNF4α in HuH7 cells increased synthesis of HBV pregenomic RNA from overlength HBV genomes under control of the naïve promoter/Enh 14-fold [53]. Deletional mapping identified two regions in coding sequences within the HBV genome (nt 1992–2435 in the core and nt 2830–3068 in the preS1 coding region) that were essential for high-level activation of pregenomic RNA synthesis by HNF4α in cis [53].

In summary, HNF4α appears to be a major regulator of pregenomic RNA transcription and thus HBV core and polymerase protein production and HBV replication. Because HNF4α is also the essential regulator of hepatocyte metabolism, development of the epithelial phenotype of hepatocytes and hepatocyte differentiation, it seems to be utilized by the virus to adapt its gene expression and replication to hepatocyte homeostasis.

Peroxisome proliferator-activated receptors (PPAR) and retinoid X receptors

Peroxisome proliferator-activated receptors are another group of nuclear hormone receptor proteins, which upon activation by a natural (fatty acids) or pharmacological (clofibric acid) ligands heterodimerize with the liver X receptor or RXR and bind to peroxisome proliferator hormone response elements. Activation of RXRα by retinoic acid or its metabolites leads likewise to formation of heterodimers with PPARα. PPARα are expressed in liver, kidney, heart, muscle and adipose tissue. They play essential roles in the regulation of cellular differentiation, development and carbohydrate, lipid and protein metabolism [54].

In HepG2.1 cells, Raney *et al.* [37] found that PPARα-RXRα heterodimers increase transcription from the preS1 the core, and the Enh I/X promoters approximately four-, four- and twofold, respectively, whereas it did stimulate the preS2/S promoter. Overexpression of PPARγ-RXRα in HuH7 cells resulted in stimulation of pregenomic RNA transcription, whereas it did not influence pre-C RNA expression [52]. Overexpression of HNF4α and RXRα plus PPARα in cells of nonhepatic origin was sufficient to initiate

transcription of the HBV 3.5-kb RNA and viral replication [55]. Analysis of PPARα knock-out, HBV-transgenic mice indicated that under physiological conditions, PPARα did not influence transcription of HBV genes. However, upon treatment with peroxisome proliferators, HBV transcripts increased resulting in increased viral replication especially in female mice [56].

Farnesoid X receptor (FXR)

Farnesoid X receptor is a liver-enriched transcription factor activated by bile acids recognizing hormone response elements by forming heterodimers with RXRα. FXR is expressed at high levels in the liver and intestine and has been shown to regulate metabolism of bile acids, lipids and gluconeogenesis. Using electrophoretic mobility shift assays, Ramiere *et al.* [57] demonstrated that FXRα-RXRα heterodimers can bind to HBV Enh II and core promoter. In HuH7 cells, bile acids enhanced activity of luciferase-reporter constructs containing the HBV Enh II and core promoter sequences through FXRα. Using a 1.3-fold overlength HBV genome, FXRα increased synthesis of the pregenomic RNA and DNA replication.

Fetoprotein transcription factor (FTF)/liver receptor homolog-1 (LRH-1)

Fetoprotein transcription factor or LRH-1 was described as a specific regulator of the α1-fetoprotein gene during early liver development and in response to hormonal signals [58]. Li *et al.* [59] cloned FTF from a human liver cDNA library and found that FTF binds to and transactivates Enh II of HBV. Using DNA–protein-binding assays, Gilbert *et al.* [60] showed that the HBV core promoter contains two high-affinity FTF-binding sites and a third lower-affinity site shared with other nuclear receptors. In transient co-transfection experiments, FTF potently activated the HBV core promoter, acting synergistically with HNF4α in the stimulation of pregenomic RNA transcription. Cai *et al.* [61] demonstrated an additional synergism between FTF and HNF1 in activating Enh II sufficient to initiate viral gene transcription and DNA replication in nonhepatic HeLa cells.

Small heterodimer partner (SHP)

Small heterodimer partner is an orphan nuclear receptor lacking a DNA-binding domain. Transfection of a plasmid encoding SHP into HepG2 cells inhibited synthesis of HBV 3.5-kb RNA and viral replication in a dose-dependent manner. In the nonhepatoma cell line 293T, SHP was shown to inhibit initiation of 3.5-kb RNA transcription largely via interaction with HNF4α. Here, synthesis of HBV pregenomic RNA was repressed to a greater extent compared to pre-C RNA [62].

Testicular orphan receptor 4 (TR4)

Testicular orphan receptor 4 was shown to bind to the direct repeat 1 sequence element (1757–1769) on the HBV core promoter and inhibit its activity in the context of an HBV genome. This resulted in suppression of HBV pre-C and pregenomic RNAs transcription. Further dissection of the inhibitory mechanism provided evidence that TR4 represses the HBV core promoter via inhibition of HNF4α-mediated transactivation by protein–protein interactions without blocking HNF4α–DNA binding [63].

Chicken ovalbumin upstream promoter transcription factor (COUP-TF)

Chicken ovalbumin upstream promoter transcription factors are members of the steroid/thyroid hormone receptor superfamily [64]. They are ubiquitous transcription factors abundant in a variety of tissues including liver and play important roles in regulating organogenesis, neurogenesis and cell differentiation [65]. In HepG2 cells, Garcia *et al.* [50] showed that COUP-TF1 binds to and antagonizes transcriptional activation mediated by the HBV Enh I in a dose-dependent manner. Later, COUP-TF1 was described to bind to the core promoter and to decrease transcriptional activation caused by HNF4α [37]. COUP-TF1 repressed synthesis of HBV pre-C and pregenomic RNA 10-fold [52,53] and caused four- and twofold reduction in synthesis of S and L RNAs, respectively, from a wild-type HBV genome [53]. Thus, COUP-TF1 indirectly inhibits HBV RNA transcription by competing with other nuclear receptors, e.g. HNF4α, for binding.

Peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC-1α)

Peroxisome proliferator-activated receptor-γ coactivator 1-α, which was cloned from a brown fat cDNA library, is a transcriptional coactivator of nuclear receptors [66]. It interacts with and co-activates a variety of nuclear receptors e.g. HNF4α or PPARs in various tissues [67]. PGC-1α synergizes with HNF4α in the regulation of carbohydrate and lipid metabolism and gluconeogenesis [68] and coordinates adaptation to metabolic changes in the liver [69]. Shlomai *et al.* [70] demonstrated that any increase in intracellular levels of PGC-1α resulted in a significant induction of HBV transcription in a dose-dependent manner. HNF4α was shown to be an important but not the sole target for HBV co-activation by PGC-1α: FOXO1 was identified as another interaction partner. *In vivo* in HBV-transgenic mice, PGC-1α was shown to adapt HBV gene transcription upon starvation [71].

Taken together, nuclear hormone receptors play an essential role in the regulation of pregenome transcription and therefore viral replication. Interactions between different

members of the nuclear hormone receptor family and PGC-1α adapt transcription from the HBV core promoter to various hormonal and metabolic stimuli. Moreover, nuclear hormone receptors represent a critical determinant of HBV liver tropism.

HBV GENE TRANSCRIPTION AND HEPATOCYTE DIFFERENTIATION

Hepatocyte differentiation is a multi-step process during which developing hepatocytes undergo a series of morphological and functional changes. It is governed by a number of liver-enriched transcription factors [72]. HBV gene expression and virus replication is only observed in differentiated, but not in undifferentiated, human hepatoma cells [73] and is more efficient in quiescent as in proliferating hepatocytes [74]. The level of HBV gene expression and replication is increased by cultivating infected primary hepatocytes [75–77] or stable HBV-producing hepatoma cell lines [75,78–80] under differentiation conditions.

Because HBV seemed to depend on hepatocyte differentiation, further studies tested whether the activity of HBV promoters and/or enhancers is regulated in a differentiation-dependent manner. Reporter assays with constructs encoding individual HBV promoters and/or enhancers showed that the activity of HBV capsid [81], preS1 and preS2/S promoters [82] was significantly higher in differentiated than in undifferentiated hepatoma cells. Raney *et al.* demonstrated that within the HBV genome context, the core promoter was two- to 20-fold and the preS1 promoter five- to 90-times more active in differentiated than in undifferentiated hepatoma cell lines [83].

Thus, the activity of HBV promoters and enhancers, regulated by a number of ubiquitous and liver-enriched transcription factors, seemed to limit expression of HBV genes to differentiated hepatocytes. In our recent work [80], we have demonstrated that HNF4α in concert with HNF1α links HBV replication to hepatocyte differentiation because it determines the efficiency of HBV pregenome transcription which is essential to initiate HBV replication.

In vivo, in HBV-transgenic mice, we analysed starting point and dynamics of viral replication during foetal liver development as well as responsible hepatocellular factors. Interestingly, HBV replication only started after birth (Quasdorff M, Nierhoff D, Protzer U, unpublished results). Although HBV 3.5-kb RNA was detected at embryonic day (ED) 12.5, its level comprised only about 1% of that detected in adult livers. Transcription of HBV genes increased during later foetal liver development, and HBV core protein became detectable at ED 18.5 with sensitive single-cell immunostaining. L-protein expression only started on day 0.5 after birth. Western blot analysis detected transcription factor COUP-TF at ED15.5, HNF1α and HNF4α at ED18.5 and PGC-1α at day 0.5 after birth. COUP-TF, which negatively regulates HBV pregenome transcription, readily increased until ED18.5, whereas positive regulators HNF1α, HNF4α and PGC-1α steadily raised determining late foetal and postnatal liver development. This interplay explains the late start of HBV transcription and replication during liver development.

A recent study by *Li et al.* [84] emphasizes the central role of transcription factor HNF4α for expression of HBV transcripts during the postnatal liver development under normal physiological conditions by breeding HBV-transgenic mice with a conditional HNF4α knock-out in hepatocytes. From birth to two weeks of age, HNF4α positive HBV-transgenic mice showed a 10-fold and fivefold increase in levels of HBV 3.5-kb RNA and HNF4α, respectively, whereas their siblings exhibiting a major reduction in HNF4α levels showed drastically reduced levels of HBV RNA transcription.

Guidotti *et al.* reported that HBV gene expression and viral replication per cell remained constant after partial hepatectomy in livers of HBV-transgenic mice [85]. This does not argue against a strong dependence of HBV gene transcription and viral replication within a highly differentiated hepatocyte, because liver regeneration after partial hepatectomy takes place by proliferation of highly differentiated hepatocytes without shortening of the G1-phase [86] or reduction in HNF4α expression levels [87].

Thus, transcriptional regulation of HBV promoters by liver-enriched transcription factors HNF4α, HNF1α and PGC-1α makes the virus susceptible to differentiation status of its host cell.

HBV TRANSCRIPTION AS POTENTIAL TARGET FOR ANTIVIRAL DRUGS

Because transcription of HBV genes is controlled by ubiquitous and liver-enriched transcription factors that are also key regulators of hepatocyte function and morphology, therapeutic down-regulation seemed hardly feasible. However, some herbal compounds, which target liver-enriched but also ubiquitous transcription factors, efficiently inhibited HBV transcription without major hepatotoxicity.

In a recent study by Hoesel *et al.*, we demonstrated that IL-6, which is well known to have hepatoprotective effects, controls HBV replication at the level of transcription. IL-6 controls expression of HNF1α and HNF4α, the two major transcription factors determining HBV promoter activity, by activating mitogen-activated protein kinases JNK and ERK and thus regulates transcription of HBV pregenomic, but also subgenomic RNAs [88].

Ying *et al.* [89] studied an antiviral effect of helioxanthin analogue 8-1 in hepatoma cells stably producing HBV. Helioxanthin analogue 8-1 was found to suppress HBV RNA and protein expression as well as DNA replication of both wild-type and 3TC-resistant virus. Further analysis revealed that the drug inhibited HBV promoter activity by decreasing HNF4α and HNF3β levels. HNF4α and HNF3β were affected post-transcription, whereas stability of neither HBV RNAs

nor proteins was influenced. The effect occurred mainly in virus-harbouring cells, indicating that HBV may sensitize the cells and the drug may selectively eliminate infected hepatocytes.

The plant *Phyllanthus amarus* was shown to inhibit HBV RNA transcription *in vitro* but also exhibited therapeutic potential in chronic HBV carriers. Ott *et al.* [90] found that the drug specifically represses HBV Enh I activity by decreased binding of C/EBP to HBV DNA.

Metformin, a drug widely used for treatment of diabetes mellitus type II, was shown to induce a protein-threonine kinase, LKB1 [91], and by this reduce levels of PGC-1α. Because PGC-1α is an important co-activator of HNF4α-mediated transcription, metformin may have antiviral potential. However, experimental prove is lacking.

C-AMP-response element binding protein is important for optimal activity all HBV promoters and enhancers [28,29]. Decoy oligonucleotides competitively binding CREB in a sequence-specific manner were able to prevent interactions between CREB and its HBV target sequences [29] without obvious toxicity.

Kim *et al.* [92] demonstrated that *Curcuma longa linn* extract has antiviral activity in HepG2 2.15 cells stably producing HBV. It decreases levels of HBV RNAs, suppresses expression of HBV antigens and production of viral particles. The extract leads to accumulation of p53 protein by enhancing transcription and stability of p53 as a potential mechanism. Oltipraz (4-methyl-5-(2-pyrazynyl)-1,2-dithiole-3-thione) is able to prevent, inhibit and even reverse carcinogenic processes. In addition, Oltipraz inhibited HBV replication dose-dependently and specifically blocked HBV transcription most likely also via the induction of p53 [93].

Taken together, targeting liver-enriched or ubiquitous transcription factors represents an interesting novel approach for HBV therapy that could help to overcome the problem of viral drug resistance. However, results are still preliminary, and toxicity of candidate compounds needs to be determined in animal models.

ACKNOWLEDGEMENTS

Basic work included in this review was supported by the Deutsche Forschungsgemeinschaft (DFG) SFB 670/576, by Koeln Fortune and by the Doerenkamp Stiftung.

REFERENCES

1 Summers J, Mason WS. Replication of the genome of a hepatitis B – like virus by reverse transcription of an RNA intermediate. *Cell* 1982; 29(2): 403–415.
2 Bock CT, Kubicka S, Manns MP, Trautwein C. Two control elements in the hepatitis B virus S-promoter are important for full promoter activity mediated by CCAAT-binding factor. *Hepatology* 1999; 29(4): 1236–1247.
3 Guidotti LG, Matzke B, Schaller H, Chisari FV. High-level hepatitis B virus replication in transgenic mice. *J Virol* 1995; 69(10): 6158–6169.
4 Wieland SF, Spangenberg HC, Thimme R, Purcell RH, Chisari FV. Expansion and contraction of the hepatitis B virus transcriptional template in infected chimpanzees. *Proc Natl Acad Sci USA* 2004; 101(7): 2129–2134.
5 Seeger C, Mason WS. Hepatitis B virus biology. *Microbiol Mol Biol Rev* 2000; 64(1): 51–68.
6 Seitz S, Urban S, Antoni C, Bottcher B. Cryo-electron microscopy of hepatitis B virions reveals variability in envelope capsid interactions. *EMBO J* 2007; 26(18): 4160–4167.
7 Chen HS, Kaneko S, Girones R *et al.* The woodchuck hepatitis virus X gene is important for establishment of virus infection in woodchucks. *J Virol* 1993; 67(3): 1218–1226.
8 Zoulim F, Saputelli J, Seeger C. Woodchuck hepatitis virus X protein is required for viral infection in vivo. *J Virol* 1994; 68(3): 2026–2030.
9 Nowock J, Borgmeyer U, Puschel AW, Rupp RA, Sippel AE. The TGGCA protein binds to the MMTV-LTR, the adenovirus origin of replication, and the BK virus enhancer. *Nucleic Acids Res* 1985; 13(6): 2045–2061.
10 Shaul Y, Ben-Levy R, De-Medina T. High affinity binding site for nuclear factor I next to the hepatitis B virus S gene promoter. *EMBO J* 1986; 5(8): 1967–1971.
11 Spandau DF, Lee CH. Repression of the hepatitis B virus enhancer by a cellular factor. *J Gen Virol* 1992; 73(Pt 1): 131–137.
12 Ori A, Atzmony D, Haviv I, Shaul Y. An NF1 motif plays a central role in hepatitis B virus enhancer. *Virology* 1994; 204(2): 600–608.
13 Krainc D, Bai G, Okamoto S *et al.* Synergistic activation of the N-methyl-D-aspartate receptor subunit 1 promoter by myocyte enhancer factor 2C and Sp1. *J Biol Chem* 1998; 273(40): 26218–26224.
14 Raney AK, Le HB, McLachlan A. Regulation of transcription from the hepatitis B virus major surface antigen promoter by the Sp1 transcription factor. *J Virol* 1992; 66(12): 6912–6921.
15 Raney AK, McLachlan A. Characterization of the hepatitis B virus large surface antigen promoter Sp1 binding site. *Virology* 1995; 208(1): 399–404.
16 Zhang P, Raney AK, McLachlan A. Characterization of functional Sp1 transcription factor binding sites in the hepatitis B virus nucleocapsid promoter. *J Virol* 1993; 67(3): 1472–1481.
17 Li J, Ou JH. Differential regulation of hepatitis B virus gene expression by the Sp1 transcription factor. *J Virol* 2001; 75(18): 8400–8406.
18 Hess J, Angel P, Schorpp-Kistner M. AP-1 subunits: quarrel and harmony among siblings. *J Cell Sci* 2004; 117(Pt 25): 5965–5973.
19 Choi BH, Park CJ, Rho HM. Insulin activates the hepatitis B virus X gene through the activating protein-1 binding site in HepG2 cells. *DNA Cell Biol* 1998; 17(11): 951–956.
20 Lee DH, Choi BH, Rho HM. The synergistic transactivation of the hepatitis B viral (HBV) pregenomic promoter by the E6 protein of human papillomavirus type 16 (HPV-16 E6) with HBV X protein was mediated through the AP1 site of E

element in the enhancer I (EnI) in human liver cell. *Biochem Biophys Res Commun* 1999; 265(1): 62–66.
21 Kornberg RD. The molecular basis of eukaryotic transcription. *Proc Natl Acad Sci USA* 2007; 104(32): 12955–12961.
22 Bogomolski-Yahalom V, Klein A, Greenblat I, Haviv Y, Tur-Kaspa R. The TATA-less promoter of hepatitis B virus S gene contains a TBP binding site and an active initiator. *Virus Res* 1997; 49(1): 1–7.
23 Qin J, Gao DM, Jiang QF *et al.* Prospero-related homeobox (Prox1) is a corepressor of human liver receptor homolog-1 and suppresses the transcription of the cholesterol 7-alpha-hydroxylase gene. *Mol Endocrinol* 2004; 18(10): 2424–2439.
24 Qin J, Zhai J, Hong R *et al.* Prospero-related homeobox protein (Prox1) inhibits hepatitis B virus replication through repressing multiple cis regulatory elements. *J Gen Virol* 2009; 90(Pt 5): 1246–1255.
25 Herzig S, Hedrick S, Morantte I, Koo SH, Galimi F, Montminy M. CREB controls hepatic lipid metabolism through nuclear hormone receptor PPAR-gamma. *Nature* 2003; 426(6963): 190–193.
26 Herzig S, Long F, Jhala US *et al.* CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature* 2001; 413(6852): 179–183.
27 Zhang B, Liu S, Perpetua MD, Walker WH, Harbrecht BG. Cytokines increase CRE binding but decrease CRE-mediated reporter activity in rat hepatocytes by increasing c-Jun. *Hepatology* 2004; 39(5): 1343–1352.
28 Tacke F, Liedtke C, Bocklage S, Manns MP, Trautwein C. CREB/PKA sensitive signalling pathways activate and maintain expression levels of the hepatitis B virus pre-S2/S promoter. *Gut* 2005; 54(9): 1309–1317.
29 Kim BK, Lim SO, Park YG. Requirement of the cyclic adenosine monophosphate response element-binding protein for hepatitis B virus replication. *Hepatology* 2008; 48(2): 361–373.
30 Gilmore TD. Introduction to NF-kappaB: players, pathways, perspectives. *Oncogene* 2006; 25(51): 6680–6684.
31 Lin YC, Hsu EC, Ting LP. Repression of hepatitis B viral gene expression by transcription factor nuclear factor-kappaB. *Cell Microbiol* 2009; 11(4): 645–660.
32 Tokusumi Y, Zhou S, Takada S. Nuclear respiratory factor 1 plays an essential role in transcriptional initiation from the hepatitis B virus x gene promoter. *J Virol* 2004; 78(20): 10856–10864.
33 Costa RH, Kalinichenko VV, Holterman AX, Wang X. Transcription factors in liver development, differentiation, and regeneration. *Hepatology* 2003; 38(6): 1331–1347.
34 Chen M, Hieng S, Qian X, Costa R, Ou JH. Regulation of hepatitis B virus ENI enhancer activity by hepatocyte-enriched transcription factor HNF3. *Virology* 1994; 205(1): 127–132.
35 Li M, Xie Y, Wu X, Kong Y, Wang Y. HNF3 binds and activates the second enhancer, ENII, of hepatitis B virus. *Virology* 1995; 214(2): 371–378.
36 Raney AK, Zhang P, McLachlan A. Regulation of transcription from the hepatitis B virus large surface antigen promoter by hepatocyte nuclear factor 3. *J Virol* 1995; 69(6): 3265–3272.
37 Raney AK, Johnson JL, Palmer CN, McLachlan A. Members of the nuclear receptor superfamily regulate transcription from the hepatitis B virus nucleocapsid promoter. *J Virol* 1997; 71(2): 1058–1071.
38 Tang H, McLachlan A. Mechanisms of inhibition of nuclear hormone receptor-dependent hepatitis B virus replication by hepatocyte nuclear factor 3beta. *J Virol* 2002; 76(17): 8572–8581.
39 Cirillo LA, Lin FR, Cuesta I, Friedman D, Jarnik M, Zaret KS. Opening of compacted chromatin by early developmental transcription factors HNF3 (FoxA) and GATA-4. *Mol Cell* 2002; 9(2): 279–289.
40 Schrem H, Klempnauer J, Borlak J. Liver-enriched transcription factors in liver function and development. Part I: the hepatocyte nuclear factor network and liver-specific gene expression. *Pharmacol Rev* 2002; 54(1): 129–158.
41 Lopez-Cabrera M, Letovsky J, Hu KQ, Siddiqui A. Transcriptional factor C/EBP binds to and transactivates the enhancer element II of the hepatitis B virus. *Virology* 1991; 183(2): 825–829.
42 Lopez-Cabrera M, Letovsky J, Hu KQ, Siddiqui A. Multiple liver-specific factors bind to the hepatitis B virus core/pregenomic promoter: trans-activation and repression by CCAAT/enhancer binding protein. *Proc Natl Acad Sci USA* 1990; 87(13): 5069–5073.
43 Choi BH, Park GT, Rho HM. Interaction of hepatitis B viral X protein and CCAAT/enhancer-binding protein alpha synergistically activates the hepatitis B viral enhancer II/pregenomic promoter. *J Biol Chem* 1999; 274(5): 2858–2865.
44 Odom DTZN, Gordon DB, Bell GW *et al.* Control of pancreas and liver gene expression by HNF transcription factors. *Science* 2004; 303(5662): 1311–1312.
45 Pontoglio M, Barra J, Hadchouel M *et al.* Hepatocyte nuclear factor 1 inactivation results in hepatic dysfunction, phenylketonuria, and renal Fanconi syndrome. *Cell* 1996; 84(4): 575–585.
46 Kimata T, Nagaki M, Tsukada Y, Ogiso T, Moriwaki H. Hepatocyte nuclear factor-4alpha and -1 small interfering RNA inhibits hepatocyte differentiation induced by extracellular matrix. *Hepatol Res* 2006; 35(1): 3–9.
47 Raney AK, Easton AJ, Milich DR, McLachlan A. Promoter-specific transactivation of hepatitis B virus transcription by a glutamine- and proline-rich domain of hepatocyte nuclear factor 1. *J Virol* 1991; 65(11): 5774–5781.
48 Zhou DX, Yen TS. The ubiquitous transcription factor Oct-1 and the liver-specific factor HNF-1 are both required to activate transcription of a hepatitis B virus promoter. *Mol Cell Biol* 1991; 11(3): 1353–1359.
49 Raney AK, Eggers CM, Kline EF *et al.* Nuclear covalently closed circular viral genomic DNA in the liver of hepatocyte nuclear factor 1 alpha-null hepatitis B virus transgenic mice. *J Virol* 2001; 75(6): 2900–2911.
50 Garcia AD, Ostapchuk P, Hearing P. Functional interaction of nuclear factors EF-C, HNF-4, and RXR alpha with hepatitis B virus enhancer I. *J Virol* 1993; 67(7): 3940–3950.
51 Yu X, Mertz JE. Promoters for synthesis of the pre-C and pregenomic mRNAs of human hepatitis B virus are genetically distinct and differentially regulated. *J Virol* 1996; 70(12): 8719–8726.

52 Yu X, Mertz JE. Differential regulation of the pre-C and pregenomic promoters of human hepatitis B virus by members of the nuclear receptor superfamily. *J Virol* 1997; 71(12): 9366–9374.
53 Yu X, Mertz JE. Distinct modes of regulation of transcription of hepatitis B virus by the nuclear receptors HNF4alpha and COUP-TF1. *J Virol* 2003; 77(4): 2489–2499.
54 Michalik L, Auwerx J, Berger JP *et al.* International Union of Pharmacology. LXI. Peroxisome proliferator-activated receptors. *Pharmacol Rev* 2006; 58(4): 726–741.
55 Tang H, McLachlan A. Transcriptional regulation of hepatitis B virus by nuclear hormone receptors is a critical determinant of viral tropism. *Proc Natl Acad Sci USA* 2001; 98(4): 1841–1846.
56 Guidotti LG, Eggers CM, Raney AK *et al.* In vivo regulation of hepatitis B virus replication by peroxisome proliferators. *J Virol* 1999; 73(12): 10377–10386.
57 Ramiere C, Scholtes C, Diaz O *et al.* Transactivation of the hepatitis B virus core promoter by the nuclear receptor FXRalpha. *J Virol* 2008; 82(21): 10832–10840.
58 Galarneau L, Pare JF, Allard D *et al.* The alpha1-fetoprotein locus is activated by a nuclear receptor of the Drosophila FTZ-F1 family. *Mol Cell Biol* 1996; 16(7): 3853–3865.
59 Li M, Xie YH, Kong YY, Wu X, Zhu L, Wang Y. Cloning and characterization of a novel human hepatocyte transcription factor, hB1F, which binds and activates enhancer II of hepatitis B virus. *J Biol Chem* 1998; 273(44): 29022–29031.
60 Gilbert S, Galarneau L, Lamontagne A, Roy S, Belanger L. The hepatitis B virus core promoter is strongly activated by the liver nuclear receptor fetoprotein transcription factor or by ectopically expressed steroidogenic factor 1. *J Virol* 2000; 74(11): 5032–5039.
61 Cai YN, Zhou Q, Kong YY *et al.* LRH-1/hB1F and HNF1 synergistically up-regulate hepatitis B virus gene transcription and DNA replication. *Cell Res* 2003; 13(6): 451–458.
62 Oropeza CE, Li L, McLachlan A. Differential inhibition of nuclear hormone receptor-dependent hepatitis B virus replication by the small heterodimer partner. *J Virol* 2008; 82(8): 3814–3821.
63 Lin WJ, Li J, Lee YF *et al.* Suppression of hepatitis B virus core promoter by the nuclear orphan receptor TR4. *J Biol Chem* 2003; 278(11): 9353–9360.
64 Wang LH, Ing NH, Tsai SY, O'Malley BW, Tsai MJ. The COUP-TFs compose a family of functionally related transcription factors. *Gene Expr* 1991; 1(3): 207–216.
65 Pereira FA, Tsai MJ, Tsai SY. COUP-TF orphan nuclear receptors in development and differentiation. *Cell Mol Life Sci* 2000; 57(10): 1388–1398.
66 Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* 1998; 92(6): 829–839.
67 Lin J, Handschin C, Spiegelman BM. Metabolic control through the PGC-1 family of transcription coactivators. *Cell Metab* 2005; 1(6): 361–370.
68 Yoon JC, Puigserver P, Chen G *et al.* Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature* 2001; 413(6852): 131–138.
69 Puigserver P, Spiegelman BM. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. *Endocr Rev* 2003; 24(1): 78–90.
70 Shlomai A, Paran N, Shaul Y. PGC-1alpha controls hepatitis B virus through nutritional signals. *Proc Natl Acad Sci USA* 2006; 103(43): 16003–16008.
71 Shlomai A, Shaul Y. The metabolic activator FOXO1 binds hepatitis B virus DNA and activates its transcription. *Biochem Biophys Res Commun* 2009; 381(4): 544–548.
72 Watt AJ, Garrison WD, Duncan SA. HNF4: a central regulator of hepatocyte differentiation and function. *Hepatology* 2003; 37(6): 1249–1253.
73 Le Jossic C, Glaise D, Corcos L *et al.* trans-Acting factors, detoxication enzymes and hepatitis B virus replication in a novel set of human hepatoma cell lines. *Eur J Biochem* 1996; 238(2): 400–409.
74 Ozer A, Khaoustov VI, Mearns M *et al.* Effect of hepatocyte proliferation and cellular DNA synthesis on hepatitis B virus replication. *Gastroenterology* 1996; 110(5): 1519–1528.
75 Galle PR, Hagelstein J, Kommerell B, Volkmann M, Schranz P, Zentgraf H. In vitro experimental infection of primary human hepatocytes with hepatitis B virus. *Gastroenterology* 1994; 106(3): 664–673.
76 Galle PR, Schlicht HJ, Kuhn C, Schaller H. Replication of duck hepatitis B virus in primary duck hepatocytes and its dependence on the state of differentiation of the host cell. *Hepatology* 1989; 10(4): 459–465.
77 Schulze-Bergkamen H, Untergasser A, Dax A *et al.* Primary human hepatocytes–a valuable tool for investigation of apoptosis and hepatitis B virus infection. *J Hepatol* 2003; 38(6): 736–744.
78 Gripon P, Diot C, Corlu A, Guguen-Guillouzo C. Regulation by dimethylsulfoxide, insulin, and corticosteroids of hepatitis B virus replication in a transfected human hepatoma cell line. *J Med Virol* 1989; 28(3): 193–199.
79 Glebe D, Berting A, Broehl S *et al.* Optimised conditions for the production of hepatitis B virus from cell culture. *Intervirology* 2001; 44(6): 370–378.
80 Quasdorff M, Hosel M, Odenthal M *et al.* A concerted action of HNF4alpha and HNF1alpha links hepatitis B virus replication to hepatocyte differentiation. *Cell Microbiol* 2008; 10(7): 1478–1490.
81 Zhang P, McLachlan A. Differentiation-specific transcriptional regulation of the hepatitis B virus nucleocapsid gene in human hepatoma cell lines. *Virology* 1994; 202(1): 430–440.
82 Chang HK, Ting LP. The surface gene promoter of the human hepatitis B virus displays a preference for differentiated hepatocytes. *Virology* 1989; 170(1): 176–183.
83 Raney AK, Milich DR, Easton AJ, McLachlan A. Differentiation-specific transcriptional regulation of the hepatitis B virus large surface antigen gene in human hepatoma cell lines. *J Virol* 1990; 64(5): 2360–2368.
84 Li L, Oropeza CE, Sainz B Jr, Uprichard SL, Gonzalez FJ, McLachlan A. Developmental regulation of hepatitis B virus biosynthesis by hepatocyte nuclear factor 4alpha. *PLoS ONE* 2009; 4(5): e5489.

85 Guidotti LG, Matzke B, Chisari FV. Hepatitis B virus replication is cell cycle independent during liver regeneration in transgenic mice. *J Virol* 1997; 71(6): 4804–4808.
86 Fausto N, Campbell JS. The role of hepatocytes and oval cells in liver regeneration and repopulation. *Mech Dev* 2003; 120(1): 117–130.
87 Flodby P, Antonson P, Barlow C, Blanck A, Porsch-Hallstrom I, Xanthopoulos KG. Differential patterns of expression of three C/EBP isoforms, HNF-1, and HNF-4 after partial hepatectomy in rats. *Exp Cell Res* 1993; 208(1): 248–256.
88 Hosel M, Quasdorff M, Wiegmann K *et al.* Not interferon, but interleukin-6 controls early gene expression in hepatitis B virus infection. *Hepatology* 2009; 50(6): 1773–1782.
89 Ying C, Li Y, Leung CH, Robek MD, Cheng YC. Unique antiviral mechanism discovered in anti-hepatitis B virus research with a natural product analogue. *Proc Natl Acad Sci USA* 2007; 104(20): 8526–8531.
90 Ott M, Thyagarajan SP, Gupta S. Phyllanthus amarus suppresses hepatitis B virus by interrupting interactions between HBV enhancer I and cellular transcription factors. *Eur J Clin Invest* 1997; 27(11): 908–915.
91 Shaw RJ, Lamia KA, Vasquez D *et al.* The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. *Science* 2005; 310(5754): 1642–1646.
92 Kim HJ, Yoo HS, Kim JC *et al.* Antiviral effect of Curcuma longa Linn extract against hepatitis B virus replication. *J Ethnopharmacol* 2009; 124(2): 189–196.
93 Chi WJ, Doong SL, Lin-Shiau SY, Boone CW, Kelloff GJ, Lin JK. Oltipraz, a novel inhibitor of hepatitis B virus transcription through elevation of p53 protein. *Carcinogenesis* 1998; 19(12): 2133–2138.