EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

The replication cycle of hepatitis B virus

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(1) Reversible and non-cell-type specific ***attachment*** to cell-associated heparan sulfate proteoglycans.

(2) Specific and probably ***irreversible binding*** to an unknown hepatocyte-specific preS1-receptor. This step presumably requires activation of the virus resulting in exposure of the myristoylated N-terminus of the L-protein [1].

(3) Two different entry pathways have been proposed: (3A) ***endocytosis*** followed by release of nucleocapsids from endocytic vesicles; (3B) ***fusion*** of the viral envelope at the plasma membrane.

(4) Cytoplasmic ***release of the viral nucleocapsid*** containing the relaxed circular partially double stranded DNA (rcDNA) with its covalently linked polymerase.

(5) ***Transport*** of the nucleocapsid along microtubules. Accumulation of the capsids at the nuclear envelope facilitates interactions with adaptor proteins of the nuclear pore complex.

(6) Possible trapping of the nucleocapsid in the nuclear basket and ***release of rcDNA*** into the nucleoplasm. The mechanisms determining the breakdown of the capsid and the release of the viral DNA genome are unsolved [2].

(7) "***Repair***" of the incoming rcDNA: Completion of the plus strand of the rcDNA by the viral polymerase. Removal of the polymerase from the 5′-end of the minus strand DNA. Removal of a short RNA-primer used for the DNA-plus strand synthesis. Both processes are mediated by cellular enzymes [3].

(8) ***cccDNA formation*** by covalent ligation of both DNA strands (reviewed in [3]). The cccDNA molecule is organized as a chromatin-like structure displaying the typical beads-on-a string arrangement consisting of both histone and non-histone proteins (***minichromosome***) [4]. The lack of cccDNA in artificial host cells (e.g. hepatocytes of HBV transgenic mice) suggests that host specific factors may regulate cccDNA formation.

(9) ***Transcription***. The cccDNA utilizes the cellular transcriptional machinery to produce all viral RNAs necessary for protein production and viral replication. Both host transcription factors, such as CCAAT/enhancer-binding protein (C/EBP) and hepatocyte nuclear factors (HNF) and viral proteins (core, the regulatory X-protein) regulate this process [4] and may modulate viral gene expression by interacting with the viral promoters of the four major overlapping open reading frames (ORFs): (I) the precore/core gene, coding for the nucleocapsid protein and for the non-structural, secreted, precore protein, the HBeAg; (II) the polymerase gene coding for the reverse transcriptase, RNase H and terminal protein domains; (III) the L-,M-, and S-gene, coding for the three envelope proteins, which are synthesized in frame from different promoters; and (IV) the X gene, coding for the small regulatory X-protein. A correlation between viremia levels and the acetylation status of cccDNA-bound histones has been reported [5], indicating that epigenetic mechanisms can regulate the transcriptional activity of the cccDNA.

(10) All 4 major mRNAs utilize a single common ***polyadenylation*** signal. ***Processing*** of viral RNAs, ***nuclear export*** as well as ***stabilization*** of the viral RNAs appears to be exclusively mediated by host factors (i.e. La RNA binding protein).

(11) ***Translation*** of the pregenomic RNA (pgRNA) to the core protein and the viral polymerase. The regulatory X-protein and the three envelope proteins are translated from the subgenomic RNAs.

(12) ***Complex formation*** of the pgRNA (via its epsilon stem-loop structure) with the core protein and the polymerase and self-assembly of an RNA-containing nucleocapsid.

(13) ***Reverse transcription*** of the pgRNA followed by plus-strand DNA-synthesis within the nucleocapsid. ***Maturation*** of the RNA-containing nucleocapsids to DNA-containing nucleocapsids within the cytoplasm.

(14) DNA-containing nucleocapsids can be either ***re-imported into the nucleus*** to form additional cccDNA molecules (14A) or can be ***enveloped for secretion*** (14B). The envelope proteins are co-translationally inserted into the ER membrane, where they bud into the ER lumen, and are secreted by the cell, either as 22 nm subviral envelope particles (SVPs) or as 42 nm infectious virions (Dane particles) if they have enveloped the DNA-containing nucleocapsids before budding. During synthesis of the L-protein, the preS-domains remain cytoplasmically exposed and become myristoylated. At some step after preS-mediated nucleocapsid envelopment translocation across the membrane occurs.

(15) Experiments performed using duck hepatitis B revealed that the majority of cccDNA molecules in infected hepatocytes comes from newly synthesized nucleocapsids. 1–50 cccDNA molecules appear to accumulate per cell, though

Received 25 October 2009; accepted 27 October 2009

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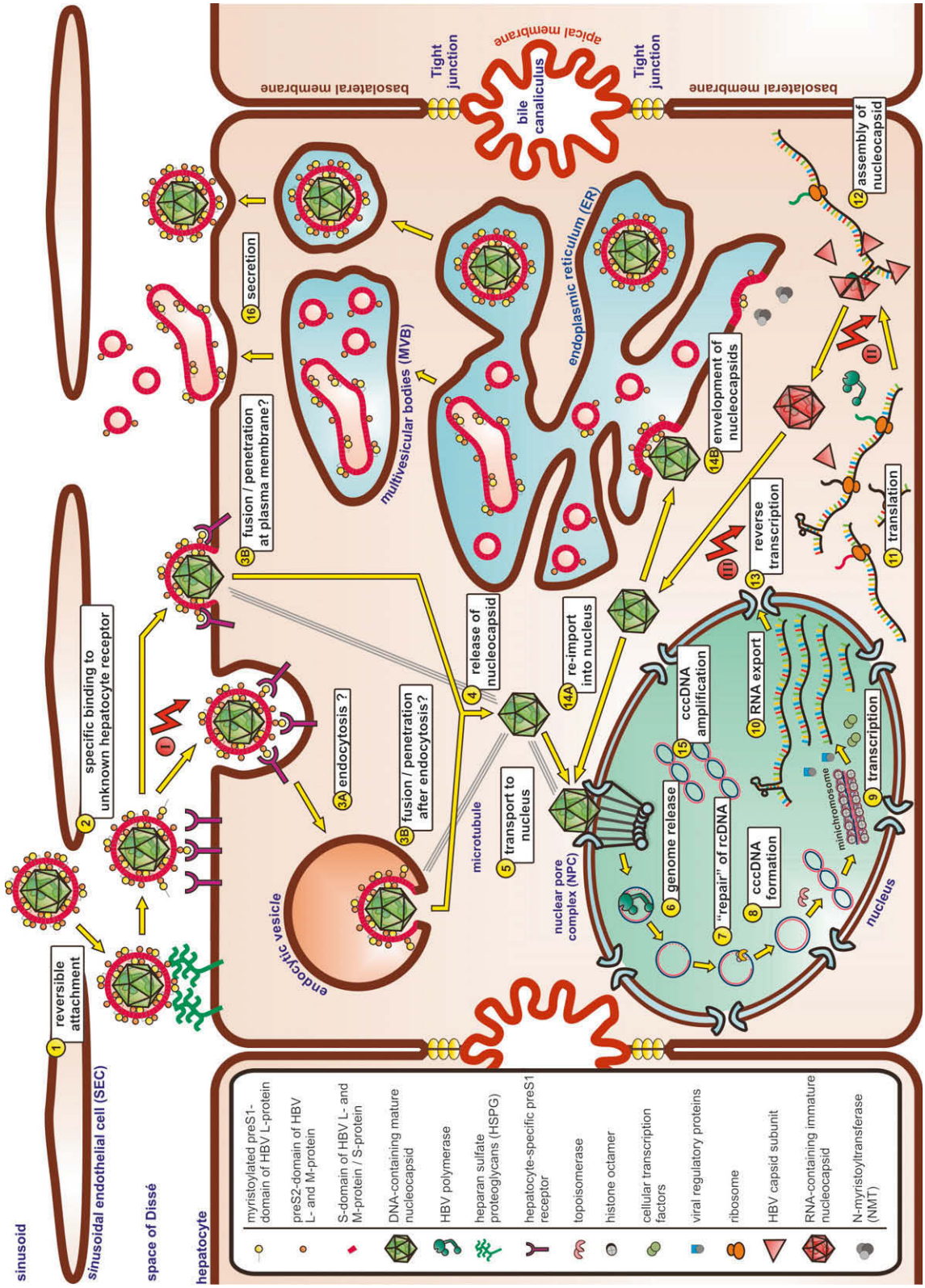
Hepatology Snapshot



Hepatology Snapshot

differences in cccDNA dynamics and efficiency of ***cccDNA accumulation*** may exist between HBV and the other hepadnaviruses. Both viral and host factors controlling cccDNA formation and pool size are yet poorly defined. A negative-feedback mechanism suppressing cccDNA amplification might involve the L-protein. As HBV polymerase inhibitors do not directly affect the cccDNA, a decrease in cccDNA levels is supposed to derive from the lack of sufficient recycling of viral nucleocapsids to the nucleus, due to inhibition of viral DNA-synthesis in the cytoplasm, and less incoming viruses from the blood [6].

(16) Compared to virions spherical and filamentous SVPs are ***secreted*** in a 10^3–10^6-fold excess into the blood of infected individuals. SVPs lack a nucleocapsid and are therefore non-infectious.

Therapeutic agents interfering with HBV life-cycle: (I) Acylated preS1-peptides have been shown to bind the HBV-receptor and block viral entry *in vivo* [7]; (II) Dihydroarylpyrimidines interfere with nucleocapsid assembly and induce core protein degradation [8]. (III) Polymerase inhibitors suppress reverse transcription and synthesis of the DNA-plus strand. The preS1-derived lipopeptides and the dihydroarylpyrimidines are presently in preclinical development. Nucleos(t)ide analogues (Lamivudine, Adefovir, Entecavir, Telbivudine, Tenofovir) and interferon (IFN) α / PEG-IFN α are the only currently approved therapeutic treatments. IFN α inhibits HBV both through immune modulatory effects and directly by reducing steady-state levels of HBV transcripts.

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