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Review

The earliest steps in hepatitis B virus infection

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Abstract

The early steps in hepatitis B virus (HBV) infection, a human hepadnavirus, initiates from cell attachment followed by entry and delivery of the genetic information to the nucleus. Despite the fact that these steps determine the virus-related pathogenesis, their molecular basis is poorly understood. Cumulative data suggest that this process can be divided to cell attachment, endocytosis, membrane fusion and post-fusion consecutive steps. These steps are likely to be regulated by the viral envelope proteins and by the cellular membrane, receptors and extracellular matrix. In the absence of animal model for HBV, the duck hepadnavirus DHBV turned out to be a fruitful animal model. Therefore data concerning the early, post-attachment steps in hepadnaviral entry are largely based on studies performed with DHBV in primary duck liver hepatocytes. These studies are now starting to illuminate the mechanisms of hepadnavirus route of cell entry and to provide some new insights on the molecular basis of the strict species specificity of hepadnavirus infection.

Keywords: Hepatitis B; Hepadnavirus; HBV infection

1. Introduction

Hepatitis B virus (HBV), a human hepadnavirus, is an enveloped DNA virus that primarily infects human hepatocytes. Viral infection may progress to acute or chronic hepatitis, and may eventually cause cirrhosis and hepatocellular carcinoma (HCC) (reviewed in Ref. [1]). The envelope of the Dane particle, the infectious virion, is made of a cell-originated membrane containing the viral envelope proteins called surface antigens (HBsAgs). The inner nucleocapsid contains the viral DNA genome, the viral polymerase/reverse transcriptase and accessory factors that originating from infected cells. In addition, the serum of the infected individuals contains non-infectious sub-viral particles that comprise over 90% of the total particles.

Hepadnavirus infection is strictly species and tissue specific therefore excluding animal models for human HBV infection studies. Several human hepatocyte cell lines retaining some of the hepatocyte markers are available that support HBV transcription and replication upon plasmid transfection. However, even these permissive cell lines are not susceptible to HBV infection. An inevitable conclusion is that the early steps of virus–cell attachment and entry determine the viral tropism and susceptibility.

The investigation of the mechanism of endocytosis and membrane fusion for HBV is at its infancy. Following endocytosis, and membrane fusion, the nucleocapsid reaches the nucleus in yet poorly identified processes. At this stage, the encased viral DNA enters the nucleus possibly via the nuclear pore complex (NPC) [2,3]. Within the nucleus, the relaxed circular viral DNA is converted to a covalently closed circular DNA (cccDNA), the template for viral gene expression and replication.

The earliest stage in HBV infection involves therefore many distinct steps from cell recognition, attachment, endocytosis, fusion and translocation of the genomic DNA to the nucleus. The number of steps is expected to increase with the fine resolution of the underlying molecular mechanisms. In this review, we will cover the recent achievements in investigating the process of hepadnavirus attachment to the target cells and the most immediate consequences.

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2. Measuring HBV–cell attachment

Attempts were made to measure HBV recognition and attachment to the existing HBV permissive hepatocyte cell lines but no significant progress was made due to the fact that these cells are refractory to HBV infection. Also, human primary hepatocytes are only variably susceptible to infection and for a short period [4–7]. To improve susceptibility, the permissive cell lines were cultivated under different conditions. Improved HBV [4,8] and DHBV infection [5,7] was obtained by the exposure of the cells to chemicals such as dimethyl sulfoxide (DMSO) and to polyethylene glycol (PEG). Combined treatment with DMSO and 100 μM 5-aza-2′-deoxycytidine further sensitized HepG2 to HBV infection (our unpublished observation) as well as avian hepatocytes to DHBV infection [9]. This protocol set the stage for measuring HBV–cell attachment.

Several methods have been described to study HBV– and DHBV–cell attachment [7,10–12]. However, due to the transient nature of the attachment process, its study and quantitative measurements turned out to be rather difficult. We have recently utilized synthetic beads with conjugated viral proteins to quantitatively evaluate virus–cell attachment at a single cell resolution by light microscopy [8]. This method permits to evaluate the attachment capacity of a given cell and to categorize or separate cells based on attachment competence. In addition to attachment, entry of the HBV–protein conjugated beads into the cells can be easily detected by scanning and transmission electron microscopy. It has been reported that envelope proteins as particles are by far much better than the soluble proteins in competing viral infection [13]. The presence of a large number of viral conjugated proteins per bead is therefore expected to improve the attachment kinetics. This improvement is in particular important for tracking epitopes with weak cell attachment activity. In addition, co-conjugation of a mixture of ligands permits detection of cooperative binding activity. Finally, use of beads that are readily visualized allows real-time analysis of cell attachment. Thus, immediate effects of viral attachment on cell behavior and morphology can be monitored.

3. The prominent HBV epitope mediating cell attachment

The HBV envelope proteins consist of the small HBsAg (SHBsAg, p24 and gp27), the middle HBsAg (MHBsAg, gp33 and gp36) and the large HBsAg (LHBsAg, p39 and gp42), all sharing the C-terminal 225 amino acids (Fig. 1). It is well documented that LHBsAg, the quantitatively minor envelope protein, plays the prominent role in cell attachment and infection of hepadnaviruses. The unique N-terminal 109 or 120 amino acids of the LHBsAg, depending on the viral subtype, named preS1, bears the major cell attachment epitope. A number of cellular proteins were identified that

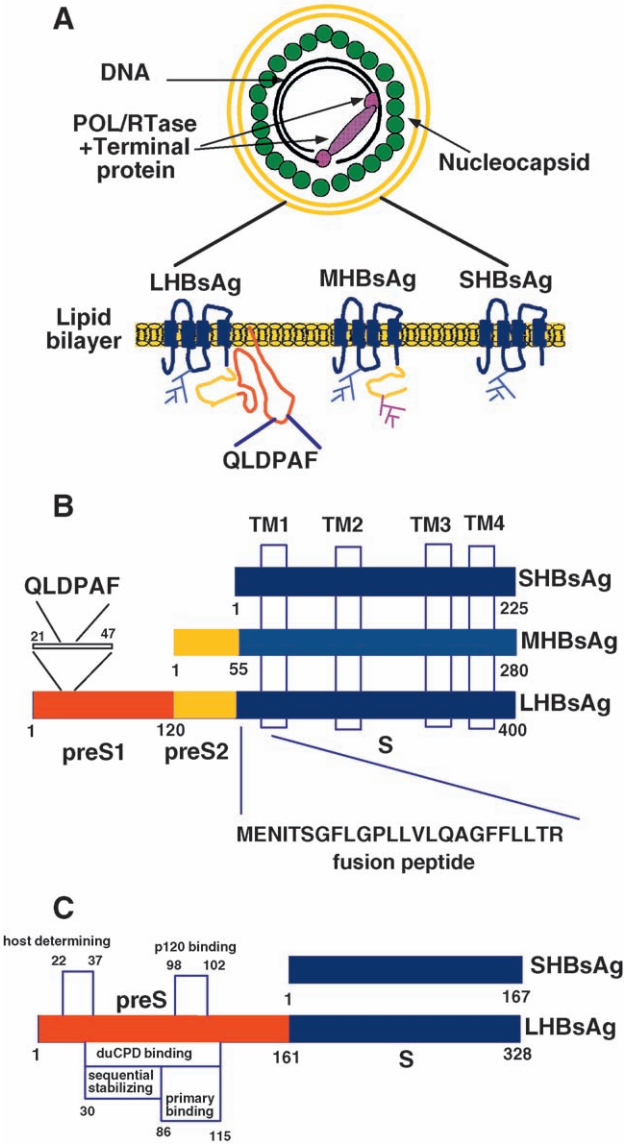

Fig. 1. General structure of the hepadnavirus and its surface proteins. (A) Schematic representation of the hepatitis B virion showing the viral DNA covalently attached to the terminal protein of the viral polymerase/reverse transcriptase (POL/RTase). This complex is enclosed by an inner nucleocapsid and an outer envelope (double layered circle). A schematic presentation of the viral membrane with the three different envelope proteins is shown. Filled boxes represent transmembrane domains of the surface proteins. Tree-like symbols represent glycosylation sites. The preS1/preS2 domains are differentially lightened. Approximate location of the QLDPAF sequence is also shown. (B) Schematic representation of HBV surface proteins. Blank boxes correspond to the transmembrane (TM) regions. The prominent HBV attachment epitope (amino acids 21–47 of the preS1 domain) containing the QLDPAF sequence is shown. Also shown is the location and sequence of the putative HBV fusion peptide. (C) Schematic representation of DHBV surface proteins. The duCPD (gp180) binding subdomain (amino acids 30–115 of the preS domain) consists of an α-helical region (amino acids 86–115) conferring primary, low-affinity binding and of a non-structured region (amino acids 30–86) that binds sequentially to generate a high-affinity complex [32]. The p120 binding site (amino acids 98–102 of preS domain) is also shown [34]. The host-determining region (amino acids 22–37 of the preS domain) renders heron HBV infectious to primary duck hepatocytes [36].

bind HBV envelope proteins, mostly to the LHBsAg [14–23]. This epitope was functionally narrowed down to amino acids 21–47 of preS1 [10]. Furthermore, by employing synthetic peptides, it was found that this epitope is not only required but also sufficient to attach specifically HepG2 cells [10,24–26]. Later by combined mutagenesis studies and single cell attachment analysis, the QLDPAF sequence within this epitope was found to be the crucial sequence for cell attachment [8].

In light of the fact that HBV cell attachment and infection are highly cell type specific, it was rather unexpected that other proteins from viral, bacterial and cellular origin share the minimal QLDPAF epitope. Most of the QLDPAF proteins have a role in cell adhesion, cell-to-cell attachment and membrane fusion [8]. This suggests that the QLDPAF sequence may have a more general role in viral and microbial infection and also in cell adherence and attachment. To reconcile the general role of this epitope with the narrow host range of HBV, one has to assume that variations in this epitope and the adjacent sequences determine tissue and species specificity of HBV. Consistent with this notion, studies on HBV infection of primary human hepatocytes revealed that an extended region within the preS1 sequence mediates infection [27].

Interestingly, the X protein (pX) of HBV bears a similar QLDPAR sequence at its N terminus. This pX region is highly conserved yet mutational studies have not yet assigned a particular function to this sequence. The similarity with the preS1 attachment domain raises the intriguing possibility that pX might be involved in cell attachment as well. We have identified two EGF-repeats containing proteins, FIBL-5 and FIBL-3, that bind a recombinant polypeptide containing the preS1 sequence and to a synthetic peptide containing the 21–47 amino acids attachment epitope. Expression of FIBL-5 is induced under conditions whereby HepG2 cells are sensitized to HBV infection in agreement with its possible involvement in mediating HBV–cell attachment (our unpublished data). Interestingly, FIBL-3 binds pX as well [28]. Thus, pX might have a role in cell attachment that so far has been overlooked.

4. Multivalent and cooperative HBV–cell attachment

Although the preS1 region contains the major cell attachment epitope, a second epitope outside the preS1 region was detected that is involved in cell attachment [8]. Particles made solely of the small HBsAg specifically attached cells albeit with a low efficiency possibly owing to the presence of yet unidentified attachment epitope. As expected, beads conjugated with the recombinant preS1 protein containing the prominent QLDPAF epitope but lacking the small HBsAg show efficient cell attachment. Interestingly, beads conjugated with particles containing the whole repertoire of the surface proteins were twice as much active compared to preS1 alone. Thus, it appears that HBV is a multivalent ligand containing at least two separate determinants that synergistically act to mediate effective cell attachment.

The number of the small HBsAg epitopes per particle is much higher than that of the preS1 QLDPAF attachment epitopes (a ratio of about 50 to 1, respectively). Also, it appears that the small HBsAg epitope recognizes a more general cell surface component, whereas the preS1 epitope binds a liver specific receptor [8]. The HBsAg epitope therefore is expected to readily attach cells; however, operative attachment is accomplished only in the presence of receptors that specifically interact with the preS1 epitope. According to this model, the HBsAg functions in scanning the appropriate target cells.

Multivalent cell attachment appears to be adopted by many viruses along evolution [29]. Upon binding to the first receptor, the searching for the second receptor becomes more efficient as it is performed in two rather than three dimensions. The virus might bind weakly to an abundant receptor through the first epitope. By making and breaking such weak bonds, the virus browses over the surface of a cell until the second epitope interacts with its cognate receptor to commence infection.

5. Duck HBV and cell attachment/infection

In the absence of animal models for HBV, the duck HBV (DHBV) turned out to be a fruitful animal model. As all hepadnaviruses exhibit some sequence similarity at the level of amino acid and share similar genome organization and virion structure, the data on DHBV may be, at least in part, relevant to HBV and vise versa. Unlike HBV, DHBV expresses only two envelope proteins from a single open reading frame (Fig. 1). The amino-terminal region of the large envelope protein (L) referred to as preS is not found in the smaller (S) protein. Upon transfection, DHBV can be propagated in human hepatoma cell lines [30], but DHBV infects only duck hepatocytes. Thus, the early events in DHBV infection including cell attachment are regulated by species-specific factors. The DHBV preS region is of 161 amino acids and is sufficient to compete out DHBV infection. Based on this behavior and other studies, it became evident that the preS region is directly involved in cell attachment and receptor recognition. In this regard, DHBV preS region is functionally similar to the preS1 region of the HBV envelope protein. No direct and quantitative measurements were performed for the capacity of the DHBV smaller envelope protein to bind cells. However, it has been demonstrated by competition analysis that the small S protein has a minor role [11].

To date, we have no information on the putative HBV receptor. The discovery of gp180 as a putative cellular receptor for DHBV was rewarding and instrumental in resolving some of the early steps in DHBV infection. gp180 is encoded by the duck carboxypeptidase D (duCPD)

gene [31] exhibiting a wide expression pattern. Studies on the interaction of the DHBV preS with duCPD revealed a relatively broad region in preS that mediates DHBV attachment, amino acid residues 30–115. This region was further subdivided into distinct preS domains with diverse affinities but all display cooperative and sequential interactions with duCPD. Based on these studies, it has been proposed that the three-dimensional structure rather than primary preS sequence determines effective receptor interaction. Initially, a primary attachment site forms a complex with the receptor that triggers the second unstructured site to adapt a high-affinity binding structure [13,32]. However, as gp180 exhibits no liver-specific expression and is incapable of rendering non-susceptible cell lines infectable [33], one has to conclude that gp180 although may be required is not sufficient for DHBV infection. Identification of additional DHBV preS binding proteins, such as p120 and p55, lends further support to this notion [34,35].

Studies of the early steps in hepadnavirus infection at the molecular level are now starting to illuminate the mechanisms underlying their strict species specificity. Specifically, the basis for host-discrimination between two of the avian hepadnaviruses, namely, the duck and the heron viruses (DHBV and HHBV, respectively) has been investigated. Primary duck hepatocytes are permissive to HHBV replication following transfection with the viral genome, but are refractory to HHBV infection [13,33,36]. Thus, host-discrimination between these viruses is believed to be determined at the level of virus entry. Accordingly, pseudotyping HHBV with DHBV envelope proteins rendered the virus infectious to primary duck hepatocytes [36]. Further analyses using subdomain pseudotyping and competition experiments excluded a predominant role for the gp180 binding subdomain of preS in host discrimination. Instead, a subdomain encompassing amino acids 20–40 of preS was assigned as the host-discriminatory determinant [36,37]. Oddly, a myristoylated peptide spanning the host-discriminatory subdomain from HHBV preS blocks infection of primary duck hepatocytes by DHBV, possibly at the level of viral attachment [37]. In light of this, the observation that HHBV does not infect primary duck hepatocytes may be explained by lack of interaction or modification (i.e., proteolysis) involving the host-discriminatory subdomain in a stage later than attachment. Host specificity among avian hepadnaviruses may be thus determined at a post-attachment, pre-transcriptional level, along the early steps of infection.

6. Uptake route for hepadnaviruses

Data concerning the early, post-attachment steps in hepadnaviral entry is largely limited to studies performed with DHBV in primary duck liver hepatocytes. Initially, efforts were largely focused on determining the pH dependency of the hepadnaviral uptake. This criterion is often used to classify viral uptake into either one of two strategies, namely, direct fusion of the viral envelope with the plasma membrane (pH independent) or a low-pH triggered fusion with the endosomal membrane following endocytosis of the viral particle (pH dependent) [38]. Studies aimed to investigate this issue with respect to hepadnaviruses all used lysosomotropic agents (e.g., ammonium chloride) but reached divergent conclusions [39–42]. This discrepancy possibly resulted from the variable experimental conditions and the different methodologies of analysis employed in these studies. Thus, despite the fact that the majority of these studies suggested a pH-independent entry for the virus [39,41,42], conclusions based on these studies should be taken with caution. Furthermore, several observations accumulated to date infer that moderately low pH possibly remaining in the presence of lysosomotropic agents [43], may still play a role in hepadnaviral entry. It has been demonstrated that DHBV uptake requires endocytosis [39,44], a feature usually associated with a low pH-triggered fusion mechanism [38]. Also, exposure of DHBV particles to low pH was shown to induce a conformational change in the viral large surface protein resulting in increased hydrophobicity of the virus surface (see below). Finally, acidic conditions were shown to enhance infectivity of protease-treated HBV particles (see below). Altogether, while a strict low pH dependency may not apply to hepadnaviruses to the same extent as for certain other viruses, low pH may still significantly facilitate entry of the virus.

Remarkably, low pH-induced conformational changes of the large surface protein may not only assist the virus in membrane fusion [43] but may also facilitate nucleocapsid release in the consecutive steps of viral entry. The large surface protein assumes either one of two major topologies in the envelope of mature virions [45–48]. In one topology, the preS domain is displayed on the virus surface while in the other, the same domain is sequestered inside the virus lumen. The internally sequestered preS domains are believed to form a matrix that interacts with the nucleocapsid [1]. It was demonstrated that exposing the virus to low pH triggers translocation of the internally sequestered preS domains onto the viral surface [46,47]. Thus, exposure of the virus to low pH in the endosome may allow dissociation of the enclosed nucleocapsid from the preS matrix, facilitating its release following membrane fusion.

The discovery of gp180 as a cellular receptor for DHBV greatly facilitated studying the intracellular traffic of the incoming virus. gp180 cycles between the trans-Golgi network (TGN) and the plasma membrane, predominantly maintaining a TGN localization at steady state [33,49]. Sorting of the protein involves passage through early and possibly late endosomes en route the TGN [44,50], and is controlled by its cytoplasmic domain that harbors a putative phosphorylation site for casein kinase [44,51,52]. The role of gp180 in post-attachment steps of DHBV entry was first demonstrated by the observation that expression of the full-

length protein in the human hepatocellular carcinoma cell line Huh7 resulted in internalization of the virus into these cells, whereas expression of a truncated form lacking its cytoplasmic domain arrested viral uptake at the cell surface [33]. This observation was in accord with the role of the gp180 cytoplasmic domain in retrieval of the protein from the plasma membrane [51,52]. Importantly, virus internalized following expression of the full-length form of gp180 retained an endosomal localization and was not delivered to the TGN [33], inferring that in susceptible hepatocytes, fusion may occur through this compartment. A subsequent study in primary duck hepatocytes showed that overexpression of gp180 mutated in the putative phosphorylation site reduced infection by DHBV, presumably due to lysosomal sorting of the virus–receptor complex [44]. Collectively, accumulative data confer a role for gp180 in early, post-attachment trafficking of the virus. It should be noted, however, that gp180 expression by itself does not confer susceptibility on otherwise non-susceptible, permissive cells [33]. Since gp180 suffices for DHBV internalization into endosomes, it is therefore likely that other, additional co-receptors are necessary for later stages of infection, possibly for membrane fusion.

A successful interaction of the major HBV–cell attachment epitope with its cognate cellular receptor has to trigger some downstream physiological changes that culminate in endocytosis. A synthetic epitope conjugated to a synthetic bead retained not only the proper cell attachment capacity but also uptake (Fig. 2). It is therefore very likely that the sole interaction with the cellular receptor sufficed to activate some downstream effectors that regulate endocytosis. Isolation and characterization of the putative HBV receptors should be of great importance to challenge this interesting possibility.

7. Membrane fusion

Based on sequence analysis, Rodriguez-Crespo et al. [53] have found a hydrophobic stretch of 23 amino acids at the N terminus of the small HBV surface (S) open reading frame that was tentatively assigned as a fusion peptide. Noticeably, the peptide overlaps the first transmembrane domain (TM1) of the surface antigen (Fig. 1). Comparative analysis revealed that the sequence of the peptide is highly conserved between different HBV subtypes and to a lesser degree, between different members of the hepadnavirus family, the substitutions being mostly conservative. Notably, the hepadnaviral sequences share elements conserved in fusion peptides of all paramyxoviruses and some retroviruses, including HIV-1, HIV-2 and SIV. Also, secondary structure prediction for the sequence located C-terminally to the putative fusion peptide implied that this region adapts an α-helical conformation that harbors bulky, hydrophobic residues at every fourth and third positions in an alternate fashion [54]. Such a heptad repeat motif is implied in formation of coiled coils typically found in viral fusion and intracellular vesicle transport (vSNARE-tSNARE) complexes [55,56].

Subsequent experimental data strongly supported a role for the candidate peptides in membrane fusion during hepadnaviral entry. The fusogenic properties of the peptides were demonstrated in vitro by means of vesicle aggregation,

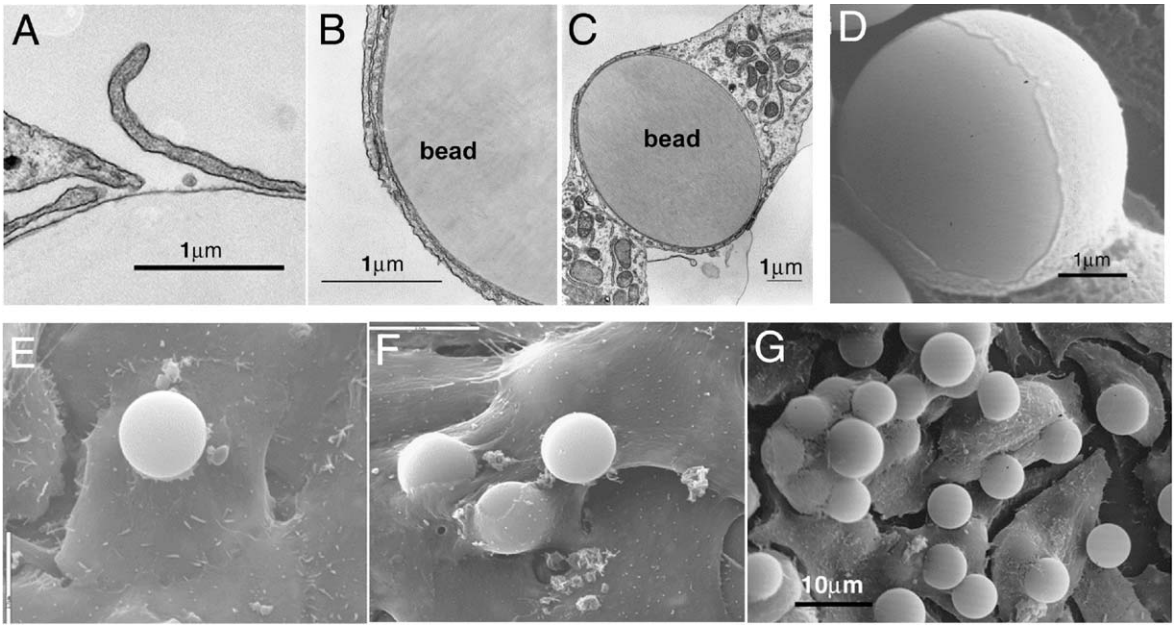

Fig. 2. Attachment and uptake of synthetic beads coated with HBV sub-viral particles (SVPs) [8]. (A–C) Transmission electron microscopy (TEM) visualization of a late stage (A) and completion (B and C) of an SVP-conjugated bead engulfment by the cell membrane. B is a higher magnification of C. (D–G) Scanning electron microscopy (SEM) visualization of different stages in attachment and uptake of SVP-conjugated beads (D–G).

lipid mixing, liposome leakage and haemolytic activity [54,57]. Pre-treating HBV and woodchuch HBV (WHV) with V8 protease that cleaves adjacent to the putative fusogenic peptides significantly enhanced the infectivity of the respective viruses in the hepatoblastoma cell line HepG2 [58,59]. Cleavage by V8 protease was suggested to expose the putative fusion peptides under the proper environmental conditions, in analogy with processing of other viruses (e.g., cleavage of the influenza HA into HA1 and HA2). Remarkably, infectivity of the protease-treated viruses was further enhanced by acidic conditions, in consistence with the notion that low pH facilitates virus entry (see above). It should be noted, however, that cleavage at this position resulted in loss of the preS domains, leading to loss of tissue specificity of the virus [60]. Therefore, in the context of viral infection in vivo, this may be settled only if a similar cleavage occurs subsequent to preS-mediated attachment of the virus to the hepatocyte cell membrane.

Studies made with DHBV offer an alternative mechanism underlying exposure of the fusogenic peptide during infection. The proposed mechanism is based on the identification of a previously unrecognized fold for the viral surface proteins in which the region connecting the two transmembrane domains (TM1 and TM2) is membrane-traversing, conferring some of the large envelope proteins with a spring-loaded, metastable structure [61]. It was found that this structure goes through a conformational change upon exposure of DHBV subviral particles to low pH, releasing TM1 onto the particle surface [43]. Release of TM1 was shown to render these particles hydrophobic based on their aggregative behavior and their liposome binding activity. Furthermore, DHBV virions were inactivated following a similar low pH pre-treatment, substantiating a role for this conformational change in the natural course of infection. Overall, these observations suggest that, similar to other viruses [62], a mechanism exists for DHBV by which the viral envelope protein is being released from a preactivated, metastable state into a fusion-active form, given the appropriate conditions. Noticeably, low pH was shown to trigger this mechanism, in accordance with its possible role in virus entry (see above).

8. Nuclear import of the viral genome

Very little information exists regarding the post-fusion events in the hepadnaviral entry. The viral genome must be transported to the nucleus, where it is transcribed. Remarkably, endocytic entry gained by the virus may facilitate its trafficking through the crowded environment of the cytoplasm and toward the nucleus [63–65]. Along the same line, to ensure productive infection, the virus likely engages a nuclear import machinery following exit from the endocytic vesicle [63]. The core particle encapsulating the viral genome is a prominent candidate for mediating post-fusion, genome nuclear transport. The core protein contains nuclear localization signals (NLSs) at its highly basic C terminus [66,67] and has been shown to bind the hepatocyte nuclear membrane [68]. *Escherichia coli*-derived core particles were shown to directly bind the NPC in a core phosphorylation- and importin-dependent manner [3]. Core phosphorylation was presumed necessary to expose C-terminally disposed, luminally sequestered NLSs onto the capsid surface. Interestingly, core phosphorylation was also shown to interfere with the nucleic acid binding activity of core [69]. Therefore, core phosphorylation may play a dual role during the initial stages of infection, promoting both the nuclear targeting and release of the viral genome. Such a model seems particularly attractive in light of the fact that the 30-nm viral capsid may be too large to transverse the nuclear pore itself. More work, however, is required to directly link the above observations to the natural delivery of the viral genome into the host nucleus.

9. Conclusions and future perspective

Attachment of HBV to target cells is the very first step in infection. A major epitope was identified that is not only required but also sufficient to mediate efficient cell attachment. The tiny genome of the virus with overlapping open reading frames makes the HBV genome one of the most compact genomes. Consequently, random sequence alteration is practically rare. As a result, the integrity of this major cell-attachment epitope, which is rather simple and readily accessible, is preserved. Despite the simple composition of this epitope, the identification of its cognate cellular receptor has faced many difficulties. Therefore, it might be that a naive model of one epitope–one receptor is invalid in this case. Indeed detailed analysis revealed that hepadnavirus attachment to the cell involves multiple components on both the cell surface and the virus envelope. The possibility that a complex of several proteins provides the functional HBV receptor should be considered. It is hoped that the recent progress in establishing cells that are effectively HBV infected and the unique power to measure quantitatively multivalent interactions in virus–cell attachment will facilitate the study and the identification of the HBV receptors. Obviously, identification of the cognate cellular receptors is instrumental to resolve the mechanism of HBV infection.

The basic rationale behind the molecular scenario of HBV infection described in this review is that HBV infects cells as a single virion. Several findings hint at the possibility that this might not be the case and HBV infects cells as a conglomerate of virions and subviral particles. According to this hypothesis, HBV-infected cells overproduce subviral particles to improve conglomerate formation and infection. The finding that subviral particles improve DHBV infection may support this notion [70]. A second supportive piece of evidence comes from the study of HBV integrants in the HCC cell line PLC/PRF/5. In these cells, a number of

independent integrants were identified that are originated from different HBV subtypes [71]. Given the fact that these cells were derived from a clonal tumor, one has to argue that a single cell experienced repeated and multiple infections. Alternatively, it is possible that all the different subtype virions were the component of a given virion conglomerate that originally infected this cell before its outgrowth as HCC. Considering the problematic issue of HBV superinfection [72], we found the second possibility a more likely one. Lastly, hepatitis delta virus (HDV) must infect and propagate in HBV-positive cells for generation of mature and infectious particles [73]. HDV is enveloped by HBV envelope proteins and therefore expected to compete with HBV infection for the very same set of receptors. This competition in infection is deleterious for HDV that its propagation depends on the envelope proteins supplemented by the coinfected HBV. Our hypothesis that infection is accomplished by virion conglomerate provides a reasonable solution to this seemingly paradoxical behavior of HDV.

Virus–cell attachment is potentially susceptible to specific inhibitors. It is possible to block either the viral attachment epitopes or the cellular cognate receptors. The former approach is believed to be less toxic to the organism and much more efficient but might be less effective for long-term treatment as escaping mutants are expected to rise. The quantitative assay for single cell attachment is expected to facilitate the screening for and the evaluation of the effectiveness of potential inhibitory drugs.

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