Hepatitis B Virus Nucleocapsid

Subjects: Virology | Gastroenterology & Hepatology

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Hepatitis B virus (HBV) is a small enveloped DNA virus which replicates its tiny 3.2 kb genome by reverse transcription inside an icosahedral nucleocapsid, formed by a single ~180 amino acid capsid, or core, protein (Cp). HBV causes chronic hepatitis B (CHB), a severe liver disease responsible for nearly a million deaths each year. Dynamic changes in Cp chemical modification and capsid conformation are crucial in the viral life-cycle and represent a promising new antiviral target.

Keywords: capsid assembly modulator (CAM) ; chronic hepatitis B (CHB) ; core protein ; HBV ; HBV cure

1. Introduction

Hepatitis B virus (HBV), the etiological agent of acute and chronic hepatitis B (CHB) in humans, is a hepatotropic small enveloped DNA virus that replicates through reverse transcription. Although its ~3 kb genome encodes only seven primary gene products (**Figure 1**) HBV is one of the most successful human pathogens. According to World Health Organization (WHO) estimates two billion people carry antibodies indicating prior exposure to the virus, and close to 300 million have become chronic virus carriers [1]. They are at a high risk to develop severe liver disease such as fibrosis, cirrhosis, and primary liver cancer [2], with nearly a million deaths per year. HBV infection can be prevented by a prophylactic vaccine yet current treatments for chronic infection are usually not curative [3]. Owing to pronounced adverse effects, only a small fraction of patients are eligible for type-I interferon (IFN) based therapies, for example, with pegylated IFN-α (pegIFNα), which after a finite 24- or 48-week treatment leads in ~10% of the patients to a sustained loss of hepatitis B surface antigen (HBsAg) [3]. Often the virus is controlled but not eliminated, a condition termed “functional cure”. Most patients are instead treated with one of the six FDA approved and better tolerated nucleos(t)ide analogs (NUCs) which inhibit reverse transcription by the multidomain HBV polymerase (P protein).

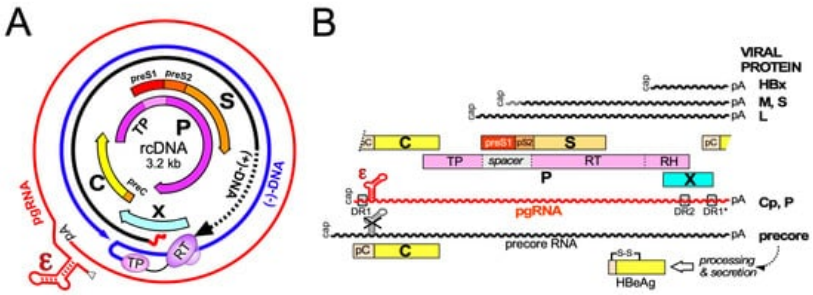

Figure 1. HBV genome organization, transcription, and translation. (**A**) Genome organization. The blue and the black line depict rcDNA with the 5′ terminally linked TP domain of P protein on the minus-strand and the RNA primer (red) on the plus-strand; the dashed black line symbolizes the incompletely filled-in 3′end. Colored internal arrows represent the ORFs with their respective preS1, preS2, and preC extensions. The outer red line depicts pgRNA with the 5′ proximal ε signal. Note that the actual transcription template is cccDNA, not rcDNA. (**B**) Viral transcripts and protein products. RNAs are shown above (subgenomic) and below (greater-than-genome length) a linear representation of the ORFs as present on the terminally redundant pgRNA; DR1, DR1*, and DR2 are direct repeats involved in rcDNA formation. As is common for eukaryotes, the most cap-proximal ORF on each RNA is translated, for precore protein enabled by the inclusion of the preC start codon in the 5′ extension; precore processing at both termini and secretion yield HBeAg. Translation of pgRNA yields Cp from the first and P protein from the second ORF; the latter initiation mechanism is still unclear.

Early NUCs were prone to rapid viral resistance development, for lamivudine in up to 70% of patients after four years of treatment [4]. The current first-line NUCs entecavir, tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide (TAF) are more potent and resistance rates are at most 1% after several years of treatment [5]. The high resistance barrier results from the massive suppression of viral genome replication, with >10 6 fold declines in serum HBV levels, and the dependence on multiple mutations for the evolution of drug-resistant yet reasonably active enzyme P protein. Still, long-

term (probably life-long) NUC therapy is necessary for most patients because, despite dampened liver inflammation, HBsAg seroclearance is rare. This is in part due to mRNA transcription from chromosomally integrated HBV DNA [6], a by-product of, but not essential for, the viral replication cycle, yet mainly to the NUCs not targeting the viral persistence reservoir, the covalently closed circular DNA (cccDNA) form of the viral genome [7]; cccDNA is produced in the host cell nucleus from the relaxed circular (rc) DNA present in infectious HB virions and is the template proper for transcription of the viral RNAs by host RNA polymerase II (see below). Hence, although formation of rcDNA containing nucleocapsids is largely blocked by NUCs, viral antigens, and immature RNA-containing nucleocapsids continue to be produced. Hence, upon therapy cessation virus replication can fully resume. As cccDNA may persist for decades, HBV reactivation can even occur long after a past self-limited acute hepatitis B [8] when immune control is lost by an unrelated disease or upon immunosuppressive treatment.

Global efforts have therefore been initiated towards new curative treatments for chronic hepatitis B, to achieve the sustained suppression of viral replication after treatment cessation, for example, functional cure, and, ideally, complete elimination of all HBV genomes from the body [9], with several intermediate distinctions [10]. Various innovative therapeutic approaches are pursued [11] which can be divided into three main categories, namely, direct acting antivirals (DAAs) targeting viral factors [12][13], inhibitors of HBV-relevant host factors [14] including the entry receptor NTCP (see below), and immune activation, aiming to restore an adequate immune response against the virus, exhaustion of which is a hallmark of CHB [2][15][16][17]. Amongst the most advanced new DAAs are the capsid assembly modulators (CAMs), also called core protein allosteric modulators (CpAMs), capsid assembly effectors (CAEs), or, very simplifying, capsid inhibitors (CIs), which target the capsid-forming HBV core protein (Cp); they are in the focus. Even though the phenotypic consequences of different CAMs may differ, their common mechanism of action (MoA) is to disable the proper dynamics of Cp and the capsid.

Excellent recent reviews on the general concept of therapeutically targeting viral structural proteins [18], analytical methods to monitor drug activities [19], and on specific CAM chemotypes [20] are available. It was intend to provide a comprehensive and comprehensible overview on the functional dynamics of HBV Cp in the viral life-cycle, on the basics of the underlying biochemical and structural dynamics, and on up-to-date clinical data on the therapeutic use of CAMs against CHB.

2. Functional Dynamics of the HBV Core Protein and Capsid in Virus Replication

The notion of viral capsid proteins is largely shaped by the highly symmetric capsid structures they can form, classically determined by X-ray crystallography (see below). Capsids provide a virus with a stable container for its genome to travel through space and time, and many structural biology techniques rely as well on the stability of these particles. However, a look on the role of capsid proteins in viral replication in general, and that of the HBV Cp in particular, reveals a highly dynamic behavior which is crucial for maintaining the viral life cycle.

Chromatinization of cccDNA by cellular histone and non-histone proteins plus viral proteins enable its regulated use as transcription template for new 5′ capped and 3′ polyadenylated viral RNAs by cellular RNA polymerase II. Eliminating cccDNA would thus truly cure infection, a highly ambitious goal [21]. Easier to realize appears to silence transcription from cccDNA which depends on the viral HBx protein. A major function of HBx is to mediate ubiquitylation and subsequent proteasomal degradation of the cellular structural maintenance of chromosomes 5/6 complex (SMC5/6) which suppresses cccDNA transcription [22]. As part of this mechanism, or in addition, HBx seems to govern intranuclear cccDNA localization which in turn contributes to its transcriptional activity [23][24]. HBx and its interactions with cellular factors have thus also become new targets for therapeutic intervention [25][26].

Electron-microscopy [27] and, as for HBx [28], chromatin immunoprecipitation (ChIP) data suggest that also some Cp is associated with cccDNA, raising the possibility that Cp contributes as well to cccDNA stability and/or transcriptional activity. A nuclear Cp interactome study identified numerous cellular RNA binding proteins, amongst them serine- and arginine-rich splicing factor 10 (SRSF10) which suppresses HBV transcription [29]; the interaction with Cp may counteract such a restriction. Conversely, Cp has been proposed to recruit cellular restriction factors of the APOBEC family to cccDNA, inducing its (partial) degradation [30]; in this view, maintaining a long-term association of Cp with cccDNA [31] could even benefit APOBEC inducing antiviral approaches. More work will be required to firmly establish cccDNA regulatory activities for Cp; it should as well be considered that the rcDNA entering the nucleus comes associated with Cp, hence ChIP assays may detect residual Cp that escaped replacement by chromatin components. Along this line, recent studies have shown that a mutant HBV defective for Cp production (itself produced by trans-complementation with a Cp expression vector) is still infectious and able to generate transcriptionally active cccDNA [32] which remained stable for over two months [33]. This also argues that the intracellular recycling pathway (see **Figure 2** step 11b), supposedly

dependent on de novo Cp synthesis and highly active for DHBV [34][35], has only a minor role for HBV cccDNA replenishment, stability, or transcriptional activity, at least in this model. Blocking recycling might then not be very effective in reducing cccDNA levels. For DHBV the more active recycling and higher cccDNA copy numbers per cell may compensate the lack of a transcription-activating HBx-like gene product, possibly via extra functions of the larger avian HBV Cp [36]. Notably, other studies on HBV concluded that stable cccDNA levels are maintained by both genome recycling and de novo secondary infections [37]. Both mechanisms would support the rapid emergence of resistant viruses during therapy with early NUCs [4][38], either by adding the mutant cccDNA to the resident wild-type (wt) cccDNA in the nucleus, or by de novo deposition in naïve cells of only mutant cccDNA. Seemingly an academic issue, determining the relative contributions of the two cccDNA pathways is crucial for future therapy design [21]. If cccDNA is mostly replenished by de novo infection, entry blockers and, possibly, also CAMs targeting the incoming nucleocapsid could effectively drain the existing cccDNA pool but would be ineffective against intracellular cccDNA recycling. The reverse holds if recycling was the dominant pathway to cccDNA maintenance. Clearly, however, the crucial initial event is specific binding of P protein to the 5′ proximal ε stem-loop structure (**Figure 2** and **Figure 3** A) on pgRNA (though not the precore RNA; [39]), with the resulting ribonucleoprotein (RNP) complex nucleating capsid shell assembly [40] by a still ill-defined mechanism.

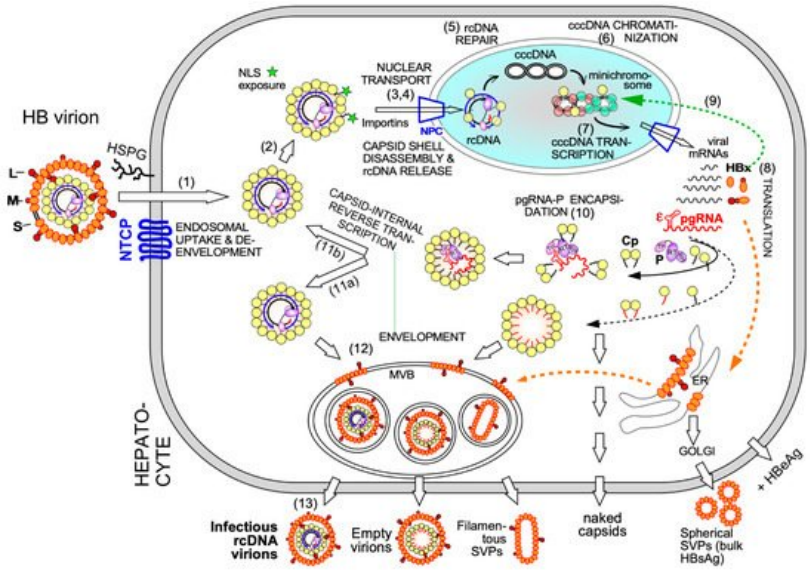

Figure 2. The HBV infectious cycle. The virion binds to heparan sulfate proteoglycans (HSPG) and via a high-affinity interaction of the L protein's PreS1 domain to the hepatocyte-specific bile acid transporter NTCP, triggering entry into the cell. The subsequent steps leading to production of infectious progeny virions are numbered. (1) Endosomal uptake and loss of the envelope; (2) exposure of NLSs to importins; (3) nuclear transport and capsid shell disassembly at the nuclear pore complex (NPC); (4) release of the rcDNA genome into the nucleus; (5) repair to cccDNA; (6) minichromosome formation; (7) transcription by RNA polymerase II; (8) translation of viral proteins, including HBx; (9) stimulation of cccDNA transcription by HBx; (10) packaging of the pgRNA-P protein complex into newly forming nucleocapsids; (11) capsid-internal reverse transcription of pgRNA into new rcDNA; via (11a) to (12) envelopment of mature rcDNA containing nucleocapsids and (13) egress from the cell via the multivesicular body (MVB) compartment; or via (11b) recycling of rcDNA (2) to the nucleus to replenish the cccDNA pool. Infected cells also release empty virions, i.e., enveloped empty capsids and small amounts of enveloped RNA containing capsids (not shown). Further viral particles include non-enveloped "naked" capsids, and spherical and filamentous subviral particles (SVPs) comprising only envelope proteins. Secreted nonparticulate HBeAg arises from processing of the precore protein (not shown).

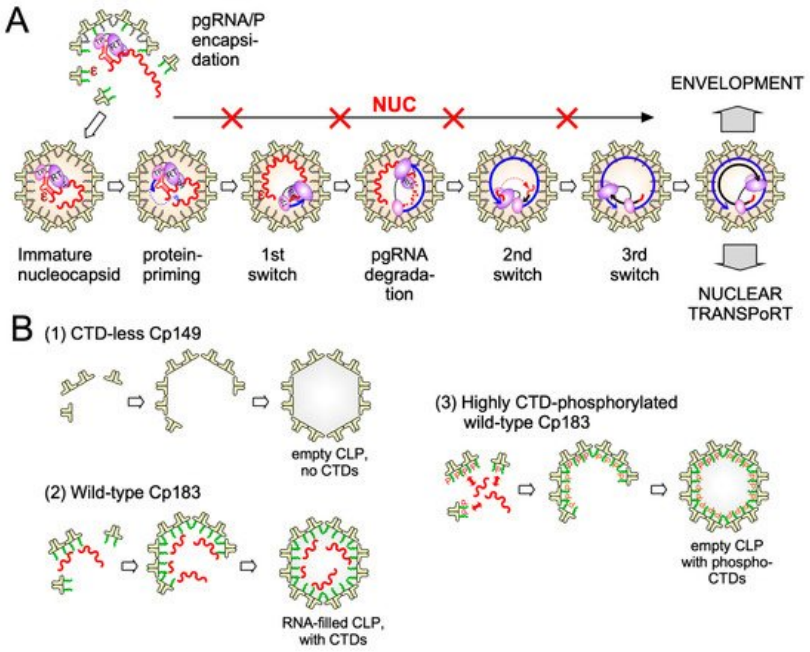

Figure 3. (**A**) The HBV capsid as distinct replication compartment. The pgRNA-P protein complex recruits Cp subunits, initiating assembly of nucleocapsids wherein pgRNA is reverse transcribed into rcDNA. P binding to ε also mediates protein-primed initiation of DNA synthesis at the ε bulge, covalently linking the TP domain to the DNA 5′ end. The short DNA oligo is transferred close to the pgRNA 3′ end (first switch) and extended towards the 5′ end, with degradation of the RNA in the nascent DNA-RNA hybrid. A 5′ terminal RNA oligo remains, is transferred to an acceptor not far from the 5′ end of the new minus-strand DNA (second switch), and primes plus-strand DNA synthesis. When reaching the template's 5′ end a third switch to the 3′ end enables circularization and further extension into mature rcDNA. NUCs inhibit DNA synthesis but not immature nucleocapsid formation. Genome maturation is assumed to induce a structural change on the capsid that signals readiness for envelopment, and also for nuclear transport as part of the recycling pathway; in neither case is the new viral DNA exposed in the cytoplasm, minimizing immune recognition as non-self. Conceivably, the entire genome maturation process is accompanied by as yet unidentified conformational alterations in the capsid. RNA is shown as red wavy line, minus-strand DNA in blue, plus-strand DNA in black. Small amounts of ds linear (dsL) DNA from a failed second template switch are not indicated. (**B**) Nonproductive assembly paths of recombinant Cps expressed in *E. coli*. In the absence of the basic C terminal domain (CTD), Cps comprising an intact N-terminal assembly domain such as Cp149 assemble empty CLP shells, essentially via Cp dimer contacts; this is the subject of most current in vitro studies. In wild-type Cp183 electrostatic interactions between the R residues in the CTD and the sugar-phosphate backbone of bacterial RNAs contribute to assembly nucleation and stabilize the CLP against inter-CTD repulsion. In highly CTD-phosphorylated Cp183 the phosphoryl groups neutralize the R residues and block their general RNA binding capacity, yielding empty, phospho-CTD containing CLPs.

3. Overall Structural Dynamics of HBV Cp

While X-ray studies can achieve resolutions down to below 1.5Å, the length of a covalent C-C bond, a major drawback is the need for crystallized samples which, in turn, is hampered by sample heterogeneity, including T = 3 vs. T = 4 dimorphism (see above). In cryo-EM, by contrast, single particles on the micrographs can be selected for image reconstruction. Averaging thousands of such particles then yields higher-resolution information, initially down to about 4Å [41], where the protein backbone and bulky sidechains became visible. The subsequent "resolution revolution" [42] brought by new detectors pushed this limit to below 3Å which reveals many, including smaller, sidechains. The currently highest resolution cryo-EM derived model for HBV capsids from full-length Cp183 (PDB 6HTX) has a nominal resolution of 2.66Å [43] but even higher resolution is principally possible [44]. Hence cryo-EM and cryo-electron tomography (cryo-ET), which generates multiple views of the same particles from different angles by specimen tilting, have become a mainstay for the study of virus structure [45].

X-ray crystallography and cryo-EM rely on sampling many identical particles; heterogeneity in the specimen will eventually cause a loss of resolution. NMR can monitor changes in the vicinity of NMR-active nuclei over a broad range of timescales [46] but is also an "ensemble technique" that reports on the average properties of a population of molecules or particles. Various less commonly known techniques are highly useful, especially in combination, to follow assembly some of which will be briefly addressed; for more information see reference [19].

A basic optical technique is light scattering which increases with particle size. Several variations exist, recently also for single-particle imaging, similar to fluorescence correlation spectroscopy. Mass spectrometry (MS) can also offer single-particle resolution, and especially charge-detection MS (CDMS) has been used to identify numerous small transitory as well as more stable “late-stage” Cp assembly intermediates, with masses between those of T = 3 and T = 4 HBV capsids [47]. Another advance are microfluidic and nanopore-based techniques, such as resistive-pulse sensing where particles of different sizes passing through the pore cause correlating changes in conductivity. Atomic force microscopy (AFM) can provide additional information on the mechanical properties of particles, including how they are affected by the material inside the particle, e.g., nucleic acid. Not the least, theoretical approaches such as molecular dynamics (MD) simulations can now handle an entire capsid [48]. A recent all-atom MD simulation of HBV capsids bound with a CAM compound suggested a mechanism for crosstalk between intra- and inter-dimer interfaces and thus a way how allostery can traverse through the entire Cp dimer [49] that is in line with experimental data [50]. Another finding was a population of dimers with significantly splayed intra-dimer interface; such opening of the four-helix bundle has also been seen in experimental studies, e.g., when capsids interact with spike-binding peptides [51], or by mutation of the spike tip-located D78 residue which, again, affects the rate of assembly [52]. While the biological meaning is not yet resolved, these convergent data exemplify how different approaches can eventually point into the same direction. Combining the different techniques can therefore provide unprecedented new insights into HBV capsid assembly as well as its inhibition; for instance, owing to the crosstalk throughout the dimer, compounds acting on the intradimer interface or the spike tip could similarly act as allosteric assembly modulators as the currently dominating CAMs that target the interdimer interface.

While new technologies promise much more detailed insights into HBV capsid assembly some basic aspects of the pathway are already established. Assembly proceeds most likely by a nucleation mechanism (comparable to the induction of crystallization from an oversaturated solution when a “crystal seed” is added), for example, the rate-limiting assembly step is the formation of an intermediate (the nucleation seed) to which additional dimeric subunits can be added (see **Figure 3**), in the elongation phase, on a downhill energetic path [53]. The likely nucleation seed for neat CTD-less Cp derivatives such as Cp149 is a three-fold symmetric trimer of dimers [54], as supported by recent EM evidence [55]; in addition, a two-fold symmetric pentamer as another predominant intermediate; there, two more dimers are associated to a trimer of dimers so as to generate two adjacent triangles. Considering that each Cp dimer is tetravalent (**Figure 4**) the trigonal trimer of dimers saturates 6 of the 12 valencies, the two-fold symmetric pentamer 12 of the totally 20 valencies; hence these arrangements minimize the number of free valencies. As assembly proceeds, the percentage of free valencies per subunit decreases further until all valencies are saturated upon addition of the last dimer. In turn, removing the first subunit from a fully assembled capsid has the highest energy cost [18], hence assembly and disassembly can be nonsymmetrical processes showing hysteresis [56] ; sophisticated techniques such as time-resolved small angle X-ray scattering confirm this view [57]. Moreover, assembly may proceed via more than one pathway, as indicated by the observation with single-particle techniques such as CDMS of particles with fewer (“defective”) or more subunits (“overgrown”) than those defining a perfect icosahedron. Both defects can slowly be corrected, suggesting that completion is a separate phase in assembly [58]. Also a recent single particle cryo-EM study found defective particles to account for a substantial fraction of the total population, perhaps reflecting that nature can live with, or even exploits, imperfection in capsid assembly [59]. The actual assembly conditions are also important for the pathways; mild conditions (including low Cp concentration and ionic strength) favor regular T = 4 CLP formation with few tangible intermediates, aggressive conditions (including high Cp concentration and ionic strength) favor formation of larger, kinetically trapped intermediates and a higher proportion of T = 3 capsids. However, the correlation of any of these in vitro conditions with assembly of replication-competent HBV nucleocapsids remains to be examined.

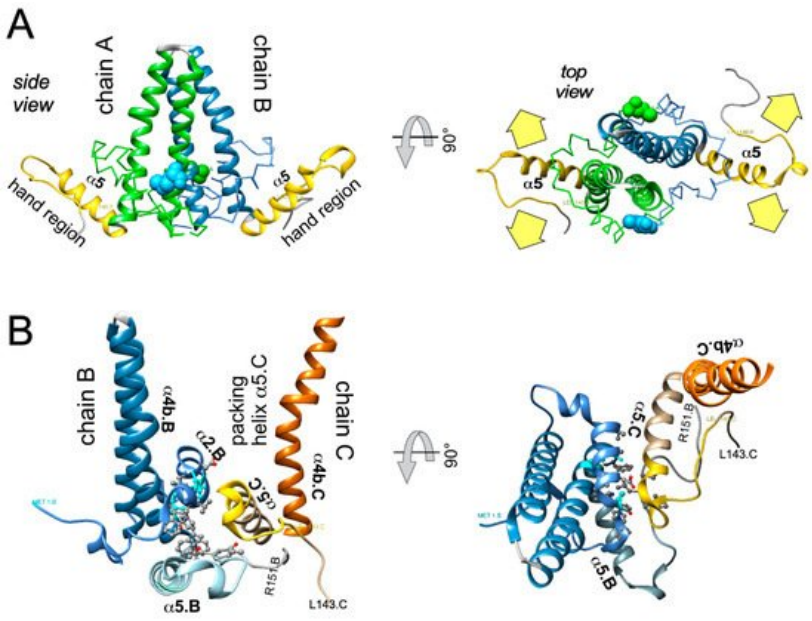

Figure 4. The HBV Cp inter-dimer interaction. (**A**) The Cp dimer is tetravalent. The “hand region” comprising helix α5, the P-rich turn and the downstream sequence to the end of the assembly domain at L140 (highlighted in gold for each monomer) is the main module for inter-dimer contacts. Each hand region provides two interfaces, hence the dimer is tetravalent, as indicated by the broad yellow arrows in the top view. Only helices α3, α4, and α5 are shown as ribbons. (**B**) Close-up of the B–C interdimer contact. For chain B the entire NTD is represented, for chain C only the descending helix α4 and the hand region. Properly oriented chain B residues from F23 through helix α2 (P25 to Y38), from α4b (W102 to F110), plus W125 and residues from P138 to S141 form a largely hydrophobic cavity, against which α5 from chain C can snuggly pack, in particular via V120, V124, W125, R127, T128, Y132, R133, P134, and P138. In the capsid, the respective residues undergo analogous interaction with their other neighbors. The pocket formed by the dimer–dimer interaction is the target for all currently known CAMs (“HAP pocket”) and the main site for CAM resistance confering mutations

4. Targeting HBV Capsid Dynamics

The two major CAM impacts on HBV nucleocapsid assembly, initially largely based on negative staining EM, are the induction of non-capsid-like aberrant multimers by CAM-A compounds, and the formation of apparently regularly shaped but genome-less capsids by CAM-E compounds; a prototypic CAM-A is the HAP BAY41-4109, a prototypic CAM-E the phenylpropenamide AT-130.

Higher resolution structural studies of CLPs with bound CAMs provided more direct information. The assumed tolerance of the capsid structure towards CAM-E compounds suggested by ensemble measurements was challenged by single particle analysis which revealed that AT-130 induced formation of empty as well as only partially completed particles [60]. In vivo such holes would dramatically change accessibility of the capsid interior to macromolecules, amongst them kinases and phosphatases, nucleases, or components of the innate immune system, all of which might represent additional layers of antiviral activity.

In sum do the biophysical data show that all current CAMs bind to essentially the same pocket at the interdimer interface; a comprehensive list of available high-resolution HBV capsid and Cp Y132A hexamer structures with bound CAMs is given in ref. [20]. They all locally stabilize the interaction and accelerate assembly, but, depending on the detailed binding mode, they can have differential impacts on the capsid structure and stability. For classic CAM-E compounds the kinetic effects seem to dominate over structural perturbations, resulting in seemingly normal capsids but overruling the role of the pgRNA-P protein complex as the natural nucleation seed (“over-initiation”). Conversely, for classic CAM-A compounds structural perturbation appears more important. However, with the discovery of ever more CAM chemotypes and derivatives thereof a whole spectrum of phenotypes can be expected which will also depend on drug concentration and binding site occupancy. All these effects should interfere with proper progression of the viral lifecycle.

Numerous CAM-A and CAM-E compounds made it into clinical trials; only a few are mentioned here. GLS4, a direct HAP successor of BAY41-4109, had initially shown less pronounced anti-HBV activity than other CAMs which could be related to its metabolization by CYP3A, one of the cytochrome P450 isoenzymes. Co-application of the CYP inhibitor ritonavir (RTV) enhanced GLS4 trough concentrations and boosted antiviral activity in phase 2 studies, with reported up to 4.4 log10 reductions in HBV DNA without added NUC [61]. Ongoing phase 2b studies evaluate the combination of GLS4/RTV

with NUC (entecavir); interim data indicate also here superiority over NUC alone, with transient ALT flares correlating with stronger antigen declines [61]. Another CAM-A HAP compound, RO7049389, also achieved up to > 3 log10 declines in HBV DNA after 28 days of treatment in phase 1 [62][63], and entered phase 2 trials evaluating long-term triple combinations, including with investigational drugs. A further drug in phase 2 is QL-007 about which little detail has been disclosed; notably, a phase 2 study of ABI-H2158, a more potent CAM-E than ABI-H0731, plus NUC has just been discontinued following elevated ALT levels in some patients (https://investor.assemblybio.com/news-releases/news-release-details/assembly-bio-announces-decision-discontinue-clinical-development ; accessed on 21 September 2021).

All direct-acting anti-infectives are prone to resistance development which also plagued early anti-HBV RT inhibitors [4]. Host-factor targeting therapies circumvent this problem but are more prone to exert adverse effects [14]. Resistance evolution requires a certain mutation rate and the production of a sufficient number of progeny genomes to generate, select, and fix the proper mutation(s) in the population. Hence a high resistance barrier relies on efficient suppression of genome replication, and a high number of mutations required to achieve resistance while not losing function. This explains the success of entecavir and tenofovir as anti-HBV NUCs. The same criteria hold for CAMs. Regarding replication suppression most CAMs still lag behind NUCs, hence elucidating the Cp sequence space that fits resistance plus proper nucleocapsid functioning is a crucial issue. As propagating HBV in the lab is still not feasible one way to address this question is structure-guided mutagenesis followed by characterization of the respective virus variants in cell culture, the other information source are sequencing data from clinical trials. The high-resolution structures of capsids with bound CAMs, as for instance those in **Figure 5**, provide detailed information on the Cp residues involved in CAM binding, and various mutational studies have functionally confirmed their relevance for resistance. For BAY41-4109 up to ~50-fold increased EC 50 values regarding HBV DNA reduction were observed for the pocket mutants D29G, Y118F and especially T33N which retained about one third the replication capacity of wt-HBV [64]. The similarity but non-identity of binding modes of different CAMs (**Figure 5**) is also reflected in their resistance profiles; for instance, P25A/S and V124F reduced susceptibility to HAP_R01 but not to the sulfamoylbenzamide SBA_R01 [65]. A new phenotyping assay facilitating comparative analyses of more mutants yielded similar results, indicating that Cp positions 33, 102, 118, and 127 affect both HAP and SBA binding while positions 25 and 109 are more important for HAP than SBA binding [66]. Also here mutation T33N conferred the highest resistance against both CAM chemotypes yet retained about half the replication capacity of wt-HBV. A comprehensive recent study probed 25 HAP pocket residues by 70 single-site substitutions for functionality and resistance to various CAM phenotypes [67]. Most replacements of W102, interacting with many CAMs, abolished Cp assembly; Cp W102Y and Cp W102H formed capsids but exerted much reduced pgRNA encapsidation and viral DNA synthesis. Hence the emergence of single-site resistance at this position, and similarly at Y132, is unlikely. Position T33 tolerated only substitutions with chemically similar residues but these include T33N. Overall, the most relevant resistance conferring mutations identified concerned position P25, T33, I105, and S106. More such studies will help to define potential escape routes for the virus and, specifically, monitor patients for dangerous variants. These will likely not be restricted to single-site mutants because, given the opportunity, even variants with severely hampered replication capacity can regain fitness by compensatory second-site mutations, as is well known from NUC resistance [4]. Notably, natural polymorphisms have been found at HAP pocket positions by searches in HBV databases [68] and a large patient cohort [66], including Y118F and D29A, I105T/L/V and T114I/V but not T33N ; the presence or absence of such baseline mutations may thus affect treatment outcome in patients, as seen by 20-fold reduced activity to AB-506 to Cp baseline mutant I105T [69]. In an early 28-day trial with JNJ-6379 half of the patients carried viruses with one or more potentially relevant mutations, including Y118F, I105T, T109M, and T019I, but their therapy response was not generally reduced, despite a detectable enrichment of the Y118F mutant [70]. Importantly, though, 24 week interim data from the JNJ-6379 monotherapy arm of the 48 week JADE study revealed viral breakthrough in several patients which correlated with baseline mutations Y118F and I105T and emergence of the T33N mutation [71]. By contrast, no viral breakthrough or enrichment of CAM resistant variants occurred in the patients from the CAM plus NUC arm of the study. Similar data were seen with ABI-H0731 monotherapy [72] versus a one-year combination treatment with ABI-H0731 plus NUC [73], corroborating the resistance-suppressing effect of more efficient replication inhibition by the drug combination.

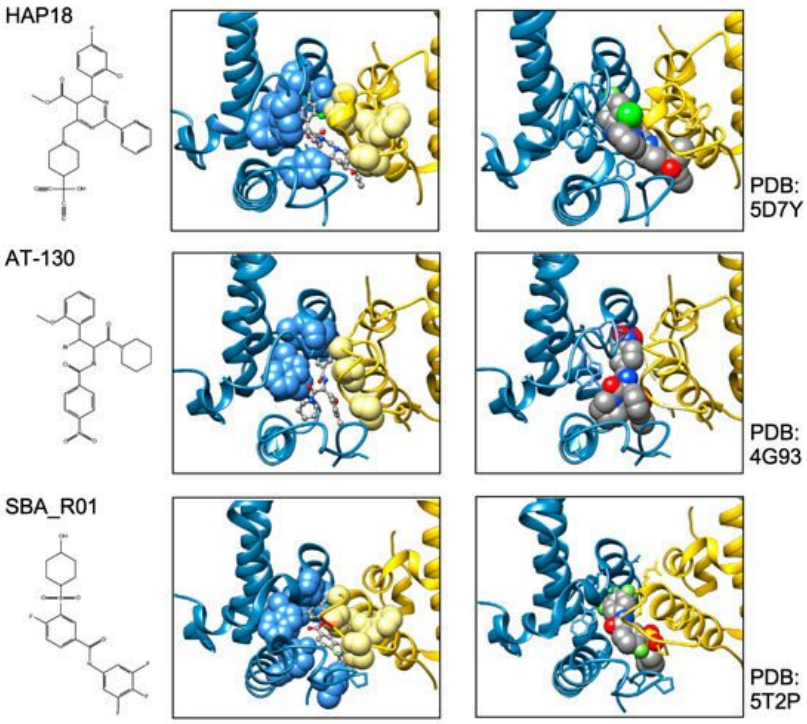

Figure 5. Different CAMs share a common binding pocket yet display non-identical binding modes. All currently known CAMs bind in the HAP pocket formed at the Cp inter-dimer interface with one Cp subunit (in blue) providing most contacts, and the second subunit (in gold) “capping” the bound compound. The compounds represent the classical chemotypes of HAP (HAP18), phenylpropenamide (AT-130) and sulfamoylbenzamidine (SBA_R01). Shown from left to right are the name and chemical formula of the respective CAM; a close-up of the HAP pocket with the most relevant protein residues as spheres and the CAM as ball-and-stick model; and the same view but some relevant protein residues as sticks and the CAM atoms as spheres. The corresponding PDB entries are given on the right. Note that despite their different chemical structure all three CAMs fit snuggly into the binding pocket yet engage both common and distinct Cp residues. This correlates with overlapping but non-identical resistance profiles and addresses different pressure points in the capsid shell which, via allostery, results in different overall responses.

5. Conclusions and Perspectives

Meanwhile small molecules targeting HBV nucleocapsid assembly have proven their ability to interfere with HBV replication in clinical settings. The efficacy of viral DNA suppression by advanced CAMs is approaching that of current first-line NUCs, hence capsid assembly modulation is clearly a valid antiviral strategy. However, as with NUCs viral rebound after cessation of CAM therapy seems common. Hence present CAMs provide a highly valuable addition but not a fundamentally superior substitute for current NUC monotherapy. Nonetheless, prospects for significant improvements are good even though more virological, pharmaceutical and clinical efforts will be needed for a final judgement. Detailed knowledge on the impact of CAMs as well as the other new treatment options on the virus, the cell, and the host organism will be the best investment for devising smart combination therapies which currently hold the greatest promise for the long-awaited HBV cure.

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