Chapter 2
HBV Genome and Life Cycle

Jie Wang, Hongxin Huang, Yongzhen Liu, Ran Chen, Ying Yan, Shu Shi, Jingyuan Xi, Jun Zou, Guangxin Yu, Xiaoyu Feng, and Fengmin Lu

Abstract Chronic hepatitis B virus (HBV) infection remains to be a serious threat to public health and is associated with many liver diseases including chronic hepatitis B (CHB), liver cirrhosis, and hepatocellular carcinoma. Although nucleos(t)ide analogues (NA) and pegylated interferon-α (Peg-IFNα) have been confirmed to be efficient in inhibiting HBV replication, it is difficult to eradicate HBV and achieve the clinical cure of CHB. Therefore, long-term therapy has been recommended to CHB treatment under the current antiviral therapy. In this context, the new antiviral therapy targeting one or multiple critical steps of viral life cycle may be an alternative approach in future. In the last decade, the functional receptor [sodium-taurocholate cotransporting polypeptide (NTCP)] of HBV entry into hepatocytes has been discovered, and the immature nucleocapsids containing the non- or partially reverse-transcribed pregenomic RNA, the nucleocapsids containing double-strand linear DNA (dslDNA), and the empty particles devoid of any HBV nucleic acid have been found to be released into circulation, which have supplemented the life cycle of HBV. The understanding of HBV life cycle may offer a new instruction for searching the potential antiviral targets, and the new viral markers used to monitor the efficacy of antiviral therapy for CHB patients in the future.

1 Introduction

Hepatitis B virus (HBV) belongs to *Hepadnaviridae* family and is the causative factor of chronic hepatitis B (CHB). Worldwide, 257 million people are chronically infected with HBV, and 887,000 people annually die of HBV infection-related end-stage liver disease, such as liver cirrhosis, liver failure, and liver cancer [1–3].

J. Wang · H. Huang · Y. Liu · R. Chen · Y. Yan · S. Shi · J. Xi · J. Zou · G. Yu
X. Feng · F. Lu (✉)
Department of Microbiology & Infectious Disease Center, School of Basic Medical Sciences, Peking University Health Science Center, Beijing, P.R. China
e-mail: lu.fengmin@hsc.pku.edu.cn

H. Tang (ed.), *Hepatitis B Virus Infection*, Advances in Experimental Medicine and Biology 1179, https://doi.org/10.1007/978-981-13-9151-4_2

Currently, there are two kinds of antiviral agents used for CHB treatment, including nucleos(t)ide analogues (NA) and pegylated interferon (Peg-IFN-α), and neither of them can directly target and efficiently clear the covalently closed circular DNA (cccDNA) which persists in the nuclei of the infected hepatocytes [4, 5]. Since cccDNA is the main cause that CHB is difficult to cure, the eradication of cccDNA is an ideal goal for the cure of CHB. Unfortunately, it is almost impossible via the currently available antiviral therapies. Alternatively, the functional cure, a state characterized with serum HBsAg loss, has been suggested. Since functional cure is also difficult to achieve, the long-term NA therapy has been recommended in almost all the guidelines for CHB management [6–9]. However, the long-term therapy may bring a series of problems, such as side effects, costs, and compliance.

Recent discoveries allow a better understanding of HBV life cycle and pave the way for identifying the multiple new therapeutic targets for CHB, as well as provide the new viral markers for guiding the clinical practice. Firstly, the HBV entry inhibitors are being developed after the discovery of NTCP as a major functional receptor of HBV infection [10–12]. Meanwhile, the approaches specifically targeting cccDNA are being explored in experimental models when the gene editing technologies discovered, such as the systems of zinc-finger nucleases, TAL effector nucleases, and CRISPR-associated (cas) nucleases [13–20]. Moreover, other antiviral agents for CHB treatment are being developed, such as the uses of RNA interference to inhibit HBV replication, capsid assembly modulators (CAMs) to inhibit pgRNA encapsidation and block HBV DNA synthesis, and immune modulatory therapies [21–29]. In the future, the combination of the current and the newly developed antiviral agents targeting the different steps of viral life cycle may be an alternative approach for achieving the eradication of HBV infection and the clinical cure of CHB. Besides, several serum viral markers, including hepatitis B core-related antigen (HBcrAg), HBV RNA, and dslDNA, are going to be the potential viral markers for monitoring the efficacy and prognosis of antiviral therapy for CHB patients [30–39].

2 HBV Genome

HBV has a genome of approximately 3.2 kb and partially double-stranded, relaxed circular DNA (rcDNA) which is composed of a complete coding minus strand (−) and an incomplete noncoding plus strand (+) with a fixed 5' end and a variable-size 3′ end [40, 41]. As shown in Fig. 2.1, the relaxed circular configuration of HBV genome is maintained by the cohesive end regions containing two direct repeats (DRs) of 11 nucleotides (TTCACCTCTGC) termed DR1 (nt 1824–1834) and DR2 (nt 1590–1600) [42]. Both DR1 and DR2 play the important roles in viral replication, and the integration of HBV DNA sequences into host cell genome [43]. The coding minus strand contains four overlapping open reading frames (ORFs) (preC/C, P, preS/S, and X), four promoters [core promoter (CP, 1613-1849) consists of the upper regulatory region (URR, nt1613-1742) and the basic core promoter

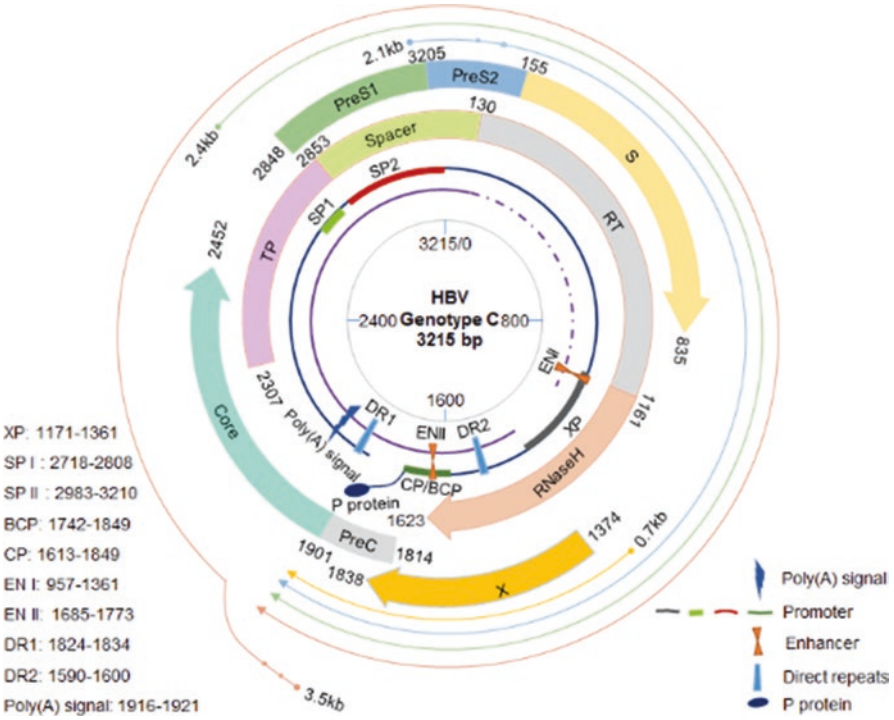

Fig. 2.1 The circular diagram of HBV genome. The locations of ORFs and important regulatory elements refer to genotype C HBV genome [45, 46]. XP, X promoter; SP I, PreS1 promoter; SP II, PreS2 promoter; BCP, Basic core promoter; CP, Core promoter; EN I, Enhancer I; EN II, Enhancer II; DR1, Direct repeat 1; DR2, Direct repeat 2; Poly(A), Polyadenylation

(BCP, nt 1742-1849), PreS1 promoter (SP I, nt 2718-2808), PreS2 promoter (SP II, nt 2983-3210), and X promoter (XP, nt 1171-1361)], and two enhancers [Enhancer I (EN I, nt 957-1361) and Enhancer II (EN II, nt 1685-1773)], as well as polyadenylation [poly(A)] signal (nt 1916-1921) [44, 45]. Under the regulation of four promoters and two enhancers, the 3.5, 2.4, 2.1, and 0.7 kb polyadenylated HBV RNAs are generated, respectively [46, 47].

The preC/C ORF and P ORF overlap each other partially: the former encodes hepatitis B e antigen (HBeAg) and core protein, and the latter is responsible for the synthesis of HBV DNA polymerase (P protein). Core protein and P protein are translated from pregenomic RNA (pgRNA), while HBeAg is translated from precore mRNA. Both pgRNA and precore mRNA are transcriptionally regulated by CP in which the URR regulates the promoter activity and the BCP regulates the transcriptions of both pgRNA and precore mRNA [48, 49]. Core protein self-assembles to form the viral capsid and binds with cccDNA to participate in its epigenetic modifications [50], while P protein consists of four pivotal domains including terminal protein (TP) domain, spacer region, reverse transcriptase (RT) domain, and

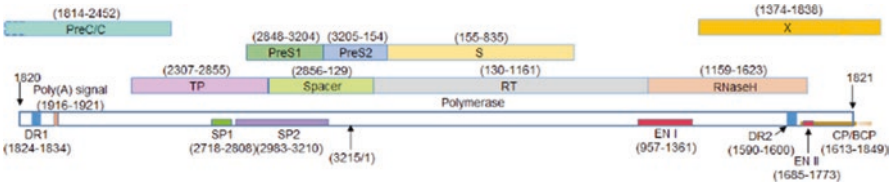

Fig. 2.2 The linear diagram of HBV genome

RNase H domain, which is anchored on the 5′ terminus of the minus-strand DNA and has many functions in the viral life cycle, such as viral RNA binding, pgRNA encapsidation, protein priming, reverse transcriptase activity, DNA polymerase activity, and RNase H activity [51–54]. The precursor of HBeAg undergoes proteolytic processing in the endoplasmic reticulum (ER) and generates the mature HBeAg [55]. Although HBeAg is not essential for the viral replication and infection, such a secretory protein has immune regulatory functions. For example, HBeAg can inhibit host innate immunity and mediate immune evasion by inducing T cell tolerance [56, 57]. The preS/S ORF is located within P ORF and uses three different in-frame AUG start codons to encode three envelope glycoproteins including large (L), middle (M), and small (S) surface antigens (HBsAg). L-HBsAg is translated from the 2.4 kb HBV RNA transcriptionally regulated by SP I, and the latter two envelope glycoproteins are translated from 2.1 kb HBV RNA transcriptionally regulated by SP II [58]. The X ORF encoding X protein (HBx) is the smallest ORF and overlaps with P ORF. HBx is translated from the 0.7 kb HBV RNA which is transcriptionally regulated by XP (Figs. 2.1 and 2.2).

In addition to the promoter elements, the expressions of these viral genes are also modulated by two enhancer elements, EN I and EN II. EN I is located between ORF S and X and is consisted of a 5′ modulatory element, a central core domain with actual enhancer activity and a 3′ domain that overlaps with X ORF [59, 60]. EN II is located at the upstream of CP and partially overlaps with BCP and URR, which comprises region IIA and IIB potent enhancer elements [47, 48]. Both EN I and EN II have the ability to upregulate the activities of the HBV promoters in an orientation-independent manner, in which EN I preferentially upregulates the activities of CP/BCP and XP but has a modest effect on the activities of SP I and SP II, while EN II preferentially upregulates the activities of the SP I, SP II, and XP [48, 49, 61, 62].

3 Viral Entry

HBV is highly species-specific and hepatotropic. This species specificity is partly dependent on the expression pattern of HBV entry receptors. As shown in Fig. 2.3, HBV has been identified to firstly attach to heparan sulfate proteoglycans (HSPGs) on hepatocyte membrane with low affinity, which is mediated by the antigenic loop (AGL) present in the S domain of all HBsAg [63]. Subsequently, PreS1 region of

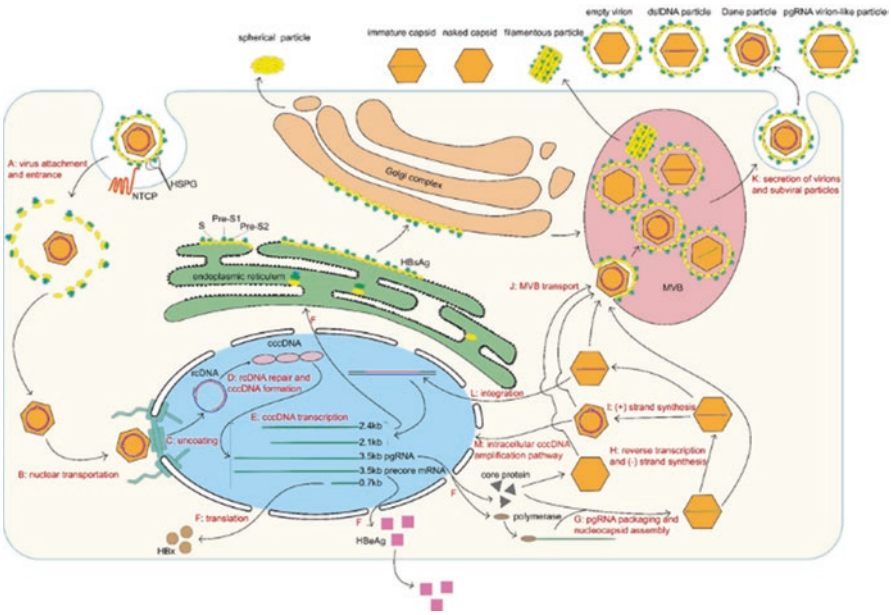

Fig. 2.3 HBV life cycle. (**a**): The entrance of HBV Dane particles into hepatocyte via the binding of L-HBsAg to NTCP and binding of S-HBsAg to HSPG on the membrane of hepatocyte. (**b**): The release of nucleocapsid and the transportation of nucleocapsid into nucleus where the uncoating takes place. (**c**): The release of rcDNA from the nucleocapsid into nucleus of hepatocyte. (**d**): The conversion of rcDNA to cccDNA which serves as the template for viral transcription. (**e**): The transcriptions of cccDNA to HBV RNAs. (**f**): The translations of HBV RNAs result in the productions of HBeAg, core protein, P protein, L-HBsAg, M-HBsAg, S-HBsAg, and HBx protein. (**g**):The binding of P protein to ε region of pgRNA and the recruitment of core proteins to assemble nucleocapsid. (**h**): The reverse transcription and the synthesis of HBV minus (−)-strand DNA using pgRNA as the template. (**i**): The accurate translocation of P protein and the synthesis of HBV plus (+)-strand DNA. (**j**): The envelopment of the nucleocapsid via MVB transport pathway. (**k**): Secretion of virions and subviral particles. (**l**): Incorrect translocation of P protein resulting in formation of dslDNA and the integration of dslDNA into the host genome. (**m**): Nuclear translocation and uncoating of newly formed nucleocapsids to replenish cccDNA pool

L-HBsAg, predominantly the 2-48 N-terminal amino acids (aa), binds to NTCP on the basolateral membrane of hepatocytes with high affinity [64]. The myristoylation of the N-terminal PreS1 facilitates virus infection through enhancing the capability of receptor recognition [65–67]. NTCP composed of 349 aa is a conjugated bile acid transporter, and the aa 84-87 and 157-165 of NTCP are critical for viral entry into hepatocytes [10, 68]. Next, the viruses are internalized through endocytosis, including the caveolae- and clathrin-mediated endocytosis [69–71]. The subsequent endosomes are translocated by a common vesicle traffic pathway relying on cytoskeleton and are regulated by Rab, small guanosine triphosphatases of the Ras superfamily, to deliver the endosomes to different cellular compartments [72, 73].

During this process, the translocation of vesicle is accompanied by a pH decrease from about 6.2 in early endosomes to approximate 5.5 in late endosomes, resulting in the fusion of the viral envelope with the endosomal membrane to release nucleocapsids [74]. However, pH-independent entry and sequential endosomal sorting seems to be the major determinants in the infection of duck hepatitis B virus (DHBV) [75]. Besides, the cholesterol on viral membrane is required for the above endosomal escape of the virus into the cytosol [76]. The nucleocapsids are transported by motor proteins along microtubules toward the nucleus [77]. The nucleocapsids are directed to nucleus pore complex (NPC) by nuclear localization sequence (NLS) of core proteins and then are arrested at the nuclear basket by Nup153, a component of NPC [78, 79]. Finally, the nucleocapsids disassemble, followed by HBV rcDNA and some core proteins releasing into nucleus [80].

4 The Conversion of rcDNA to cccDNA

Once rcDNA enters the nucleus, it will go through a series of biochemical steps to be converted to covalently closed circular DNA (cccDNA), which is the crucial intermediate that serves as the template of HBV replication during HBV life cycle (Fig. 2.3) [81]. Many host factors, such as DNA repair devices, participate in the conversion of rcDNA to cccDNA [82, 83]. Firstly, P protein anchored on the 5′ terminus of the minus-strand HBV DNA may be removed by human tyrosyl DNA phosphodiesterase-2 (TDP2) which is a host repair enzyme that can remove topoisomerase (TOP)-DNA covalent complexes [84–87]. Except for TDP2, this deproteinization reaction may also be achieved by an endonucleases-mediated nucleolytic pathway [87]. Meanwhile, a capped and 18 nucleotide-long RNA oligomer at the 5′ end of plus-strand DNA and the 9 or 10 nucleotide-long terminally redundant segment (r sequence) at the 5′ end of minus-strand DNA are removed by flap structure-specific endonuclease 1 (FEN1) which specifically cleaves the 5′-flap structure formed by RNA oligomer or r sequence [88–90]. However, other host factors other than FEN1 may also participate in removing the flap structure in rcDNA [90]. Next, the plus-strand DNA which is variable in length is extended not only by P protein but also host cellular polymerases, such as DNA polymerase κ (POLK) [88, 91, 92]. POLK is a key host cellular polymerase supporting HBV infection, while other host DNA polymerases, such as DNA polymerase L (POLL) and H (POLH), also participate in this step [92]. Finally, to converse rcDNA to cccDNA, both linear strands are mainly ligated by host cellular DNA ligase (LIG) 1 and 3 after the extension of plus-strand DNA [93]. Besides, LIG4 has been considered to participate in the formation of cccDNA from double-strand linear DNA (dslDNA) through the nonhomologous end joining (NHEJ) DNA repair pathway [93–95].

Once formed, cccDNA will be organized into a chromatin-like viral minichromosome and modified by host histone proteins, such as H3 and H4 histone proteins, as well as nonhistone proteins like viral HBx, core protein, and host epigenetics-related proteins, whereas the nucleosome spacing (repeat length) is 180 bp which is

different from the 200 bp repeat length for the chromatin of eukaryotic cells [96, 97]. HBV cccDNA can be epigenetically modified to regulate viral replication and viral gene expression, including DNA methylation and histone modifications [98].

5 The Expressions of Viral RNAs and Proteins

After rcDNA is being converted to cccDNA in the nucleus of hepatocyte, cccDNA is used as the template of HBV replication and then transcribed into the 3.5 kb, 2.4 kb, 2.1 kb, and 0.7 kb HBV RNAs [46, 81, 99–101]. As shown in Fig. 2.3, there are two kinds of the 3.5 kb HBV RNAs, the precore mRNA and pgRNA. The precore mRNA is mainly transcribed from 1783 nt of HBV genome and is longer than pgRNA which mainly transcribed from 1818 nt [102]. HBV RNAs share the same 3′ end terminus using a classic poly(A) signal "UAUAAA" (nt 1916-1921) (Fig. 2.1) [36, 38, 46]. However, there is another cryptic poly(A) signal "CAUAAA" within the X ORF (nt 1788-1793), which can lead to the productions of the truncated HBV RNAs (trRNA) [38, 103].

As mentioned above, the transcriptions of HBV RNAs are regulated by four promoters (CP, SP I, SP II, and XP) and two enhancers (EN I and EN II) [101, 104]. CP is consist of BCP and URR. BCP contains four serial TATA-like box, in which the three ahead are used to control the transcription of precore mRNA and the fourth one is used for controlling the transcription of pgRNA [105, 106]. URR is consist of a positive regulatory element (nt 1613-1636) and a negative regulatory element (1636–1742), both of which regulate the transcriptional activity of BCP [107–109]. SP I initiates the transcription of 2.4 kb mRNA, and SP II initiates the transcription of 2.1 kb mRNA [110]. XP initiates the transcription of 0.7 kb HBV RNA [111]. EN I promotes the transcriptions of precore mRNA, pgRNA and 0.7 kb HBV RNAs, but has a modest effect on the transcriptions of the 2.4 kb and 2.1 kb HBV RNAs, while EN II preferentially promotes the transcriptions of the 2.4 kb, 2.1 kb, and 0.7 kb HBV RNAs [48, 49].

HBV pgRNA can be spliced by the formation of spliceosome which could remove introns like the cellular machineries [112, 113]. Just like the intron of heterogeneous nuclear RNA (hnRNA) which is composed a 5′ donor site ("GU"), a 3′ acceptor site ("AG"), a branch site (usually "A" base), and a polypyrimidine tract [113], the deleted HBV pgRNA sequences are also mainly shown a GU-AG manner [114]. The most abundant HBV pgRNA splicing variant is termed as SP1, with nearly one third of the HBV genome deleted (from nt 2447 to 489) [112]. There are also other forms of spliced pgRNA utilizing the different 5′ donor site and 3′ acceptor site [112]. Interestingly, the spliced pgRNAs can also be encapsidated and subsequently reversed transcribed [115–118]. Moreover, SP1 can be translated into HBV splicing-generated protein (HBSP) which is reported to influence cell viability, proliferation, and migration, as well as the TNF-α signaling pathway [119–123].

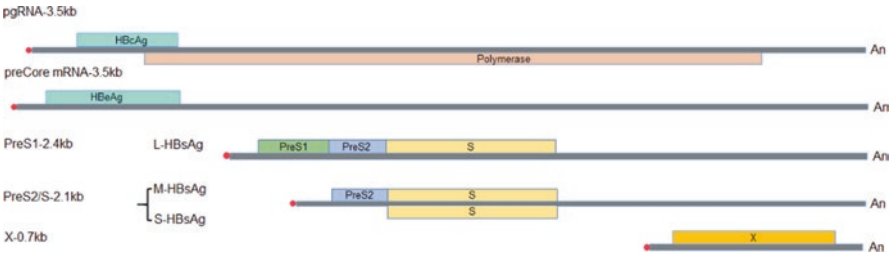

Fig. 2.4 The linear diagram of HBV RNAs and viral proteins

Subsequently, HBV RNAs are translocated into cytoplasm [99]. As shown in Fig. 2.4, the precore mRNA is firstly translated to precore polypeptide, of which the first 19 amino acids in the N-terminal region is a signal peptide trafficking precore polypeptide to the ER where the signal peptide and the 34 amino acids of C-terminal domain are removed to form the mature HBeAg [57, 124]. The mature HBeAg can be released directly into circulation [46]. Meanwhile, a part of pgRNAs are translated to core protein and P protein, and the other parts of pgRNAs are encapsulated into the nucleocapsid and serve as the templates for viral replication. The 2.4 kb HBV RNA is translated to L-HBsAg, and the 2.1 kb HBV RNA is translated to M- and S-HBsAg. Once L-, M-, and S-HBsAg are synthesized at ribosome, they will be sorted into the ER for processing and then be transferred to the Golgi apparatus for further processing. Subsequently, these HBsAg can form two kinds of subviral particles [125, 126]. The predominant subviral particles are spherical particles with a diameter of approximately 20 nm, which mainly contain S-HBsAg and are secreted via the Golgi pathway of host cells [125, 127, 128]. The other subviral particles are less numerous filamentous particles (almost 1% of the spherical particles) with a diameter of approximately 22 nm, which contain a majority of S-HBsAg proteins and equal amounts of M- and L-HBsAg [125, 127–131]. Unlike spherical particles, filamentous particles are secreted by the host cell endosomal sorting complexes required for transport (ESCRT) and the multivesicular bodies (MVB) pathway [132]. Besides, the 0.7 kb HBV RNA is translated to HBx [99, 133, 134]. HBx is a multifunctional protein that is known to activate viral and host gene transcriptions, affect DNA repair processes, as well as regulate cell growth and death [135, 136].

6 Viral Capsid Assembly, Reverse Transcription, and rcDNA Formation

P protein recognizes the epsilon (ε) stem-loop including a bulge and an apical loop near the 5′ end of pgRNA, which is the encapsidation signal of pgRNA. A P-ε ribonucleoprotein (RNP) complex is formed by structural alterations of both pgRNA and P protein [137–140]. Meanwhile, chaperones and ATP may assist the RNP complex to be the right conformation for the subsequent encapsidation, and the

priming reaction occurs at this stage [141–143]. The RNP complex is recognized and encapsidated by core protein dimers to form core particles before or after the priming reaction, and the subsequent reverse transcription occurs inside the core particles [140]. However, there may be some other packaging signal-like RNA motifs termed as preferred site (PS) for core protein binding in pgRNA, which are found by RNA SELEX assays and share a purine-rich loop recognition motif-RGAG (R = purine) [144].

The hydroxyl group of tyrosine (Tyr) in the TP domain of P protein covalently binds with the first deoxyribonucleotide in the bulge region of ε stem-loop near the 5′ end of pgRNA to initiate the reverse transcription (Fig. 2.5a) [140, 145–148]. Next, the first four (TGAA) or three nucleotides (GAA) of the nascent minus-strand DNA are originated from the bulge region of ε stem-loop, followed by translocating the oligomer covalently linked to the P protein from ε stem-loop to the DR1 at the 3′ terminus of pgRNA and leading to the elongation of minus-strand DNA (Fig. 2.5b) [149–151]. Except for the Tyr residue in TP domain, the YMDD motif in RT domain of P protein are also required for the priming activity and are important to covalently link the first deoxyribonucleotide [146–148].

It is proposed that the primer-P protein complex is arranged through a *cis*-acting element termed Phi (φ) and located between DR2 and 3′ DR1 which is complementary

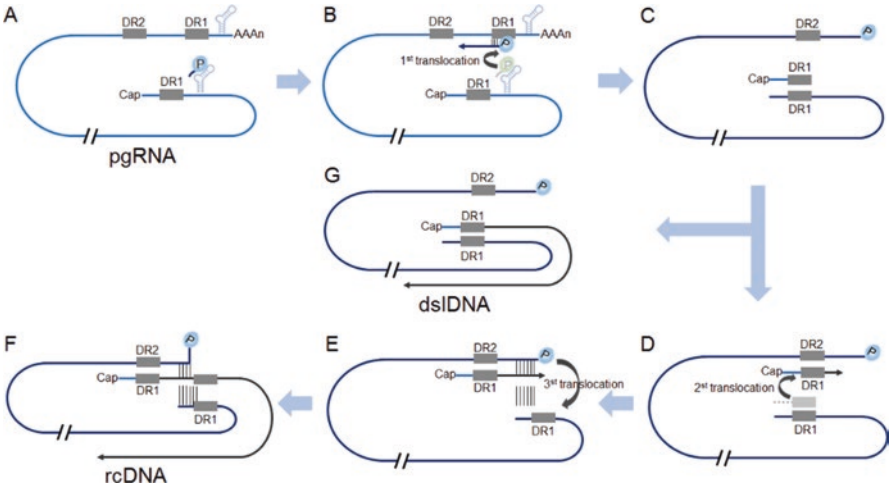

Fig. 2.5 The diagram for the formations of rcDNA and dslDNA from pgRNA. (**a**): HBV P protein-mediated priming at the ε region near the 5′ end of the pgRNA to initiate the reverse transcription process. (**b**): The first template translocation of the nascent DNA primer from ε to DR1 near the 3′ end of pgRNA. (**c**): The synthesis of minus-strand DNA and pgRNA digestion mediated by RNaseH domain of P protein. (**d**): The second template translocation of the pgRNA primer from DR1 to DR2 in the synthesis of plus-strand DNA. (**e**): The synthesis of plus-strand DNA toward 5′ end of minus-strand DNA and the third template translocation of the nascent plus-strand DNA from the 5′ end to the 3′ end of minus-strand DNA. (**f**): The formation of rcDNA in progeny virus with partial plus-strand DNA. (**g**): The formation of dslDNA through in situ priming of plus-strand DNA at the 3′ end of minus-strand DNA

to the half of 5′ ε stem-loop, thus the 5′ ε stem-loop and 3’ DRI are held in close proximity [152, 153]. In addition, another *cis*-acting element termed omega (ω) locates at the downstream of 3′ DR1 and can anneal with φ, which is also thought to be important for minus-strand DNA synthesis [154]. Following the first translocation or switch, P protein extends the minus-strand to the 5′ end of pgRNA. During the minus-strand elongation, the RNase H domain of P protein degrades pgRNA concomitantly from the pgRNA-DNA complex, whereas an oligoribonucleotide (16-18 ribonucleotides) of 5′ terminal pgRNA is reserved when the synthesis of minus-strand DNA terminated at the 5′ end of pgRNA (Fig. 2.5c) [155–157]. Notably, since the location of DR1 is within the large terminal redundancy of pgRNA, the de novo synthesized 3′ terminal minus-strand also has a terminal redundancy termed r sequence, which plays an important role in the plus-strand DNA synthesis [158].

The oligoribonucleotide of the 5′ end capped-pgRNA serves as the primer for plus-strand DNA synthesis [156, 159]. The capped-RNA oligomer (RNA primer) encompassing the DR1 sequence translocates (second switch) to the complementary DR2 sequences at the 5′ end of minus-strand and starts the synthesis of plus-strand (Fig. 2.5d) [156, 159, 160]. Once the plus-strand extends to the 5′ end of minus-strand DNA, the third translocation or switch from the 5′ end to the 3 ′ end of the minus-strand DNA will take place to continue the plus-strand DNA synthesis and form rcDNA (Fig. 2.5e and f) [54, 160]. Alternatively, without the successful second translocation, the RNA primer may remain at the 3′ end of minus-strand DNA and carry out the in situ priming of plus-DNA to form dslDNA (Fig. 2.5g) [54]. The dslDNA is preferred to be integrated into the host genome and then serves as the transcription template for HBsAg, whereas its production is a minor pathway with an occurring frequency of about 5%-20% under the normal conditions [161, 162]. Besides, several studies suggest that other *cis*-acting sequences in the minus-strand DNA may also participate to help spatially juxtapose through base pairing for plus-strand DNA synthesis [163, 164].

7 Viral Budding

The rc- or dslDNA containing core particles are termed as nucleocapsids. Some of nucleocapsids shuttle back to the nucleus to maintain a relatively stable pool of cccDNA, and other nucleocapsids are enveloped by HBV envelope glycoproteins (Fig. 2.3) [165–167]. For the latter process, two cytoplasmic domains (matrix domains, MDs) of Golgi-processed HBsAg in the MVB membrane contact with nucleocapsids, and such contacts will order the envelope proteins into a tightly packed formation in the MVB membrane and subsequently drive the inward budding process [125]. Since MD1 is located at the boundary between preS1 and preS2 in L-HBsAg (aa 103 to 124), and MD2 is located at the C-terminal half of the cytoplasmic loop between transmembrane domain (TM) 1 and 2 in S-HBsAg, it is

indicated that both L- and S-HBsAg are necessary for the inward budding while M-HBsAg is not essential [168–172]. Finally, the inward-budded nucleocapsids are sorted into the ESCRT complexes of host cell to catalyze the membrane fission and subsequently release outside the cell [131].

As shown in Fig. 2.3, except for the mature nucleocapsids, the immature nucleocapsids containing the non- or partially reverse transcribed pgRNA may also be enveloped and secreted in a similar way to the mature nucleocapsids [34, 35, 173, 174]. Besides, the empty capsids, referring to the capsids devoid of any form of HBV nucleic acid due to the core dimers failing to package HBV pgRNA, can also be enveloped by contacting with MD2 of S-HBsAg and released outside the cell as empty virions. Unlike the mature nucleocapsids, core proteins in empty capsids are mostly phosphorylated and may be the aberrant core proteins [126, 144, 175, 176].

In addition, it has been reported that the naked capsids can also be directly released outside the cell, which may be depended on the interaction between the HBV core particles and the Bro1 domain of Alix which act as a regulator of capsid releasing but independent of the ESCRT machinery (Fig. 2.3). However, the detail mechanism of this pathway has not been elucidated yet [177–179].

8 Conclusion

With an enveloped 3.2 kb rcDNA genome, HBV belongs to *Hepadnaviridae* family [180]. HBV particles enter hepatocytes through a high-affinity binding of the myristoylated viral preS1 to NTCP and a low-affinity binding of S-HBsAg to HSPG [10, 127, 181]. Subsequently, rcDNA enters into the nucleus and is converted to cccDNA, which persists as a minichromosome to transcribe HBV RNAs through the cellular transcription machinery. Among HBV RNAs, pgRNA is reverse transcribed to form HBV minus-strand DNA and encodes core protein and P protein. The assembly of viral capsid is initiated by binding of P protein to pgRNA, and then the encapsidated pgRNA is reverse transcribed to minus-strand DNA, followed by incompletely synthesizing the plus-strand DNA to form rcDNA [182]. The synthesized rcDNAs can either re-enter the nucleus to replenish cccDNA pool or be enveloped and released as viral particles [165, 183]. Except for rcDNA, dslDNA is generated when failing to translocate the RNA primer, which is often integrated into the host genome using the host enzymes [184, 185]. The integrated viral DNA fragments are frequently ended at the DR-1/2 regions of HBV genome [186, 187]. Since the intact ORF of S gene is present in the integrated viral DNA fragments, HBsAg can also be expressed from the integrated HBV DNA fragments [127, 188, 189]. Accordingly, HBsAg can be produced by either cccDNA or the integrated HBV DNA, and it has been reported that HBsAg may majorly originate from the integrated HBV DNA in HBeAg-negative HBV-infected individuals [127, 190, 191].

Like the nucleocapsids containing rcDNA, the nucleocapsids containing RNA, and the nucleocapsids containing dslDNA, the immature nucleocapsids and the empty particles may also be enveloped and released by ESCRT-dependent pathway

in MVB, which provides supplement to the traditional HBV life cycle (Fig. 2.3). These new discoveries of HBV life cycle may provide the new viral markers used for predicting the efficacy of antiviral therapy and offer the instructions for developing the new antiviral approaches.

Acknowledgment This work was supported by grants from the National S & T Major Project for Infectious Diseases (No. 2017ZX10302201) and the National Natural Science Foundation of China (No. 81672013).

References

1. GBD (2018) Causes of death collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the global burden of disease study 2017. Lancet 392(10159):1736–1788
2. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ (2015) Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. Lancet 386(10003):1546–1555
3. World Health Organization. Hepatitis B 2018 [Available from: http://www.who.int/news-room/fact-sheets/detail/hepatitis-b]
4. Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G et al (2005) Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 352(26):2682–2695
5. Wei L, Kao JH (2017) Benefits of long-term therapy with nucleos(t)ide analogues in treatment-naïve patients with chronic hepatitis B. Curr Med Res Opin 33(3):495–504
6. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ et al (2016) Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int 10(1):1–98
7. European Association for the Study of the Liver (2017) EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection. J Hepatol 67(2):370–398
8. Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM et al (2018) Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology 67(4):1560–1599
9. Omata M, Cheng AL, Kokudo N, Kudo M, Lee JM, Jia J et al (2017) Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. Hepatol Int 11(4):317–370
10. Yan H, Zhong G, Xu G, He W, Jing Z, Gao Z et al (2012) Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. elife 13:3
11. Blank A, Markert C, Hohmann N, Carls A, Mikus G, Lehr T et al (2016) First-in-human application of the novel hepatitis B and hepatitis D virus entry inhibitor myrcludex B. J Hepatol 65:483–489
12. Urban S, Bartenschlager R, Kubitz R, Zoulim F (2014) Strategies to inhibit entry of HBV and HDV into hepatocytes. Gastroenterology 147:48–64
13. Zimmerman KA, Fischer KP, Joyce MA, Tyrrell DL (2008) Zinc finger proteins designed to specifically target duck hepatitis B virus covalently closed circular DNA inhibit viral transcription in tissue culture. J Virol 82:8013–8021
14. Cradick TJ, Keck K, Bradshaw S, Jamieson AC, McCaffrey AP (2010) Zinc-finger nucleases as a novel therapeutic strategy for targeting hepatitis B virus DNAs. Mol Ther 18:947–954
15. Bloom K, Ely A, Mussolino C, Cathomen T, Arbuthnot P (2013) Inactivation of hepatitis B virus replication in cultured cells and in vivo with engineered transcription activator-like effector nucleases. Mol Ther 21:1889–1897

16. Chen J, Zhang W, Lin J, Wang F, Wu M, Chen C et al (2014) An efficient antiviral strategy for targeting hepatitis B virus genome using transcription activator-like effector nucleases. Mol Ther 22:303–311
17. Lin SR, Yang HC, Kuo YT, Liu CJ, Yang TY, Sung KC et al (2014) The CRISPR/Cas9 system facilitates clearance of the intrahepatic HBV templates in vivo. Mol Ther Nucleic Acids 3:e186
18. Zhen S, Hua L, Liu YH, Gao LC, Fu J, Wan DY et al (2015) Harnessing the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated Cas9 system to disrupt the hepatitis B virus. Gene Ther 22:404–412
19. Wang J, Xu ZW, Liu S, Zhang RY, Ding SL, Xie XM et al (2015) Dual gRNAs guided CRISPR/Cas9 system inhibits hepatitis B virus replication. World J Gastroenterol 21:9554–9565
20. Wang J, Chen R, Zhang R, Ding S, Zhang T, Yuan Q et al (2017) The gRNA-miRNA-gRNA ternary cassette combining CRISPR/Cas9 with RNAi approach Strongly Inhibits hepatitis B virus replication. Theranostics 7:3090–3105
21. Gish RG, Yuen MF, Chan HL, Given BD, Lai CL, Locarnini SA et al (2015) Synthetic RNAi triggers and their use in chronic hepatitis B therapies with curative intent. Antivir Res 121:97–108
22. Yuen M-F, Chan HLY, Liu K, Given BD, Schluep T, Hamilton J et al (2016) Differential reductions in viral antigens expressed from cccDNA vs integrated DNA in treatment naive HBeAg positive and negative patients with chronic HBV after RNA interference therapy with ARC-520. J Hepatol 64:213
23. Klumpp K, Lam AM, Lukacs C, Vogel R, Ren S, Espiritu C et al (2015) High-resolution crystal structure of a hepatitis B virus replication inhibitor bound to the viral core protein. Proc Natl Acad Sci U S A 112:15196–15201
24. Venkatakrishnan B, Katen SP, Francis S, Chirapu S, Finn MG, Zlotnick A (2016) Hepatitis B virus capsids have diverse structural responses to small-molecule ligands bound to the heteroaryldihydropyrimidine pocket. J Virol 90:3994–4004
25. Yuen M-F, Kim DJ, Weilert F, Chan HL-Y, Lalezari J, Hwang SG et al (2016) NVR 3–778, a first-in-class HBV core inhibitor, alone and in combination with peg-interferon (PegIFN), in treatment naive HBeAg-positive patients: early reductions in HBV DNA and HBeAg. J Hepatol 64:LB-06
26. Lahlali T, Berke JM, Vergauwen K, Foca A, Vandyck K, Pauwels F et al (2018) Novel potent capsid assembly modulators regulate multiple steps of the hepatitis B virus life cycle. Antimicrob Agents Chemother 62(10):pii: e00835-18
27. Lam AM, Espiritu C, Vogel R, Ren S, Lau V, Kelly M et al (2018) Preclinical characterization of NVR 3-778, a first-in-class capsid assembly modulator against hepatitis B virus. Antimicrob Agents Chemother 63(1):pii: e01734-18.
28. Sato S, Li K, Kameyama T, Hayashi T, Ishida Y, Murakami S et al (2015) The RNA sensor RIG-I dually functions as an innate sensor and direct antiviral factor for hepatitis B virus. Immunity 42:123–132
29. Korolowicz KE, Iyer RP, Czerwinski S, Suresh M, Yang J, Padmanabhan S et al (2016) Antiviral efficacy and host innate immunity associated with SB 9200 treatment in the woodchuck model of chronic hepatitis B. PLoS One 11:e0161313
30. Wang L, Cao X, Wang Z, Gao Y, Deng J, Liu X et al (2019) Correlation of HBcrAg with intrahepatic hepatitis B virus Total DNA and covalently closed circular DNA in HBeAg-positive chronic hepatitis B patients. J Clin Microbiol 57(1):pii: e01303-18
31. Loggi E, Vukotic R, Conti F, Grandini E, Gitto S, Cursaro C et al (2018) Serum hepatitis B core-related antigen is an effective tool to categorize patients with HBeAg-negative chronic hepatitis B. J Viral Hepat 26(5):568–575
32. Testoni B, Lebossé F, Scholtes C, Berby F, Miaglia C, Subic M et al (2018) Serum hepatitis B core-related antigen (HBcrAg) correlates with covalently closed circular DNA transcriptional activity in chronic hepatitis B patients. J Hepatol. pii: S0168-8278(18)32582-0

33. Liao H, Liu Y, Li X, Wang J, Chen X, Zou J et al (2018) Monitoring of serum HBV RNA, HBcrAg, HBsAg and anti-HBc levels in patients during long-term nucleoside/nucleotide analogue therapy. Antivir Ther 24(2):105–115
34. Köck J, Theilmann L, Galle P, Schlicht HJ (1996) Hepatitis B virus nucleic acids associated with human peripheral blood mononuclear cells do not originate from replicating virus. Hepatology 23:405–413
35. Jansen L, Kootstra NA, van Dort KA, Takkenberg RB, Reesink HW, Zaaijer HL (2016) Hepatitis B virus pregenomic RNA is present in virions in plasma and is associated with a response to pegylated interferon alfa-2a and nucleos(t)ide analogues. J Infect Dis 213(2):224–232
36. Wang J, Shen T, Huang X, Kumar GR, Chen X, Zeng Z et al (2016) Serum hepatitis B virus RNA is encapsidated pregenome RNA that may be associated with persistence of viral infection and rebound. J Hepatol 65(4):700–710
37. Butler EK, Gersch J, McNamara A, Luk KC, Holzmayer V, de Medina M et al (2018) Hepatitis B virus serum DNA and RNA levels in Nucleos(t)ide analog-treated or untreated patients during chronic and acute infection. Hepatology 68(6):2106–2117
38. van Bommel F, Bartens A, Mysickova A, Hofmann J, Kruger DH, Berg T et al (2015) Serum hepatitis B virus RNA levels as an early predictor of hepatitis B envelope antigen seroconversion during treatment with polymerase inhibitors. Hepatology 61:66–76
39. Zhao XL, Yang JR, Lin SZ, Ma H, Guo F, Yang RF et al (2016) Serum viral duplex-linear DNA proportion increases with the progression of liver disease in patients infected with HBV. Gut 65(3):502–511
40. Lamontagne RJ, Bagga S, Bouchard MJ (2016) Hepatitis B virus molecular biology and pathogenesis. Hepatoma Res 2:163–186
41. Glebe D, Bremer CM (2013) The molecular virology of hepatitis B virus. Semin Liver Dis 33(2):103–112
42. Nassal M (1999) Hepatitis B virus replication: novel roles for virus-host interactions. Intervirology 42(2–3):100–116
43. Wei Y, Neuveut C, Tiollais P, Buendia MA (2010) Molecular biology of the hepatitis B virus and role of the X gene. Pathol Biol (Paris) 58(4):267–272
44. Karayiannis P (2017) Hepatitis B virus: virology, molecular biology, life cycle and intrahepatic spread. Hepatol Int 11(6):500–508
45. Hao R, Xiang K, Peng Y, Hou J, Sun J, Li Y et al (2015) Naturally occurring deletion/insertion mutations within HBV whole genome sequences in HBeAg-positive chronic hepatitis B patients are correlated with baseline serum HBsAg and HBeAg levels and might predict a shorter interval to HBeAg loss and seroconversion during antiviral treatment. Infect Genet Evol 33:261–268
46. Tong S, Revill P (2016) Overview of hepatitis B viral replication and genetic variability. J Hepatol 64(1):S4–S16
47. Kim DH, Kang HS, Kim KH (2016) Roles of hepatocyte nuclear factors in hepatitis B virus infection. World J Gastroenterol 22(31):7017–7029
48. Moolla N, Kew M, Arbuthnot P (2002) Regulatory elements of hepatitis B virus transcription. J Viral Hepat 9(5):323–331
49. Quarleri J (2014) Core promoter: a critical region where the hepatitis B virus makes decisions. World J Gastroenterol 20(2):425–435
50. Zlotnick A, Venkatakrishnan B, Tan Z, Lewellyn E, Turner W, Francis S (2015) Core protein: a pleiotropic keystone in the HBV lifecycle. Antivir Res 121:82–93
51. Clark DN, Hu J (2015) Unveiling the roles of HBV polymerase for new antiviral strategies. Futur Virol 10(3):283–295
52. Ganem D, Schneider RJ (2001) Hepadnaviridae: the viruses and their replication. In: Knipe DM, Howley PM (eds) Fields virology, vol 2, 4th edn. Lippincott Williams & Wilkins, Philadelphia, pp 2923–2969

53. Jones SA, Hu J (2013) Hepatitis B virus reverse transcriptase: diverse functions as classical and emerging targets for antiviral intervention. Emerg Microbes Infect 2(9):e56
54. Nassal M (2008) Hepatitis B viruses: reverse transcription a different way. Virus Res 134(1–2):235–249
55. Venkatakrishnan B, Zlotnick A (2016) The structural biology of hepatitis B virus: form and function. Annu Rev Virol 3(1):429–451
56. Chen MT, Billaud JN, Sällberg M, Guidotti LG, Chisari FV, Jones J et al (2004) A function of the hepatitis B virus precore protein is to regulate the immune response to the core antigen. Proc Natl Acad Sci U S A 101(41):14913–14918
57. Ou JH, Laub O, Rutter WJ (1986) Hepatitis B virus gene function: the precore region targets the core antigen to cellular membranes and causes the secretion of the e antigen. Proc Natl Acad Sci U S A 83(6):1578–1582
58. Sheu SY, Lo SJ (1992) Preferential ribosomal scanning is involved in the differential synthesis of the hepatitis B viral surface antigens from subgenomic transcripts. Virology 188(1):353–357
59. Quasdorff M, Protzer U (2010) Control of hepatitis B virus at the level of transcription. J Viral Hepat 17(8):527–536
60. Huan B, Siddiqui A (1993) Regulation of hepatitis B virus gene expression. J Hepatol 17(Suppl 3):S20–S23
61. Li Y, Ito M, Sun S, Chida T, Nakashima K, Suzuki T (2016) LUC7L3/CROP inhibits replication of hepatitis B virus via suppressing enhancer II/basal core promoter activity. Sci Rep 6:36741
62. Zhang Q, Cao G (2011) Genotypes, mutations, and viral load of hepatitis B virus and the risk of hepatocellular carcinoma: HBV properties and hepatocarcinogenesis. Hepat Mon 11(2):86–91
63. Sureau C, Salisse J (2013) A conformational heparan sulfate binding site essential to infectivity overlaps with the conserved hepatitis B virus a-determinant. Hepatology 57(3):985–994
64. Glebe D, Urban S, Knoop EV, Cag N, Krass P, Grün S et al (2005) Mapping of the hepatitis B virus attachment site by use of infection-inhibiting preS1 lipopeptides and tupaia hepatocytes. Gastroenterology 129(1):234–245
65. Gripon P, Le Seyec J, Rumin S, Guguen-Guillouzo C (1995) Myristylation of the hepatitis B virus large surface protein is essential for viral infectivity. Virology 213(2):292–299
66. Bruss V, Hagelstein J, Gerhardt E, Galle PR (1996) Myristylation of the large surface protein is required for hepatitis B virus in vitro infectivity. Virology 218(2):396–399
67. De Falco S, Ruvo M, Verdoliva A, Scarallo A, Raimondo D, Raucci A et al (2001) N-terminal myristylation of HBV preS1 domain enhances receptor recognition. J Pept Res 57(5):390–400
68. Yan H, Peng B, He W, Zhong G, Qi Y, Ren B et al (2013) Molecular determinants of hepatitis B and D virus entry restriction in mouse sodium taurocholate cotransporting polypeptide. J Virol 87(14):7977–7991
69. Huang HC, Chen CC, Chang WC, Tao MH, Huang C (2012) Entry of hepatitis B virus into immortalized human primary hepatocytes by clathrin-dependent endocytosis. J Virol 86(17):9443–9453
70. Cooper A, Shaul Y (2006) Clathrin-mediated endocytosis and lysosomal cleavage of hepatitis B virus capsid-like core particles. J Biol Chem 281(24):16563–16569
71. Macovei A, Radulescu C, Lazar C, Petrescu S, Durantel D, Dwek RA et al (2010) Hepatitis B virus requires intact caveolin-1 function for productive infection in HepaRG cells. J Virol 84(1):243–253
72. Blondot ML, Bruss V, Kann M (2016) Intracellular transport and egress of hepatitis B virus. J Hepatol 64(1 Suppl):S49–S59
73. Zeyen L, Prange R (2018) Host cell Rab GTPases in hepatitis B virus infection. Front Cell Dev Biol 6:154

74. Hayes CN, Zhang Y, Makokha GN, Hasan MZ, Omokoko MD, Chayama K (2016) Early events in hepatitis B virus infection: from the cell surface to the nucleus. J Gastroenterol Hepatol 31(2):302–309
75. Funk A, Mhamdi M, Hohenberg H, Will H, Sirma H (2006) pH-independent entry and sequential endosomal sorting are major determinants of hepadnaviral infection in primary hepatocytes. Hepatology 44(3):685–693
76. Funk A, Mhamdi M, Hohenberg H, Heeren J, Reimer R, Lambert C et al (2008) Duck hepatitis B virus requires cholesterol for endosomal escape during virus entry. J Virol 82(21):10532–10542
77. Rabe B, Glebe D, Kann M (2006) Lipid-mediated introduction of hepatitis B virus capsids into nonsusceptible cells allows highly efficient replication and facilitates the study of early infection events. J Virol 80 (11): 5465–5473.
78. Li HC, Huang EY, Su PY, Wu SY, Yang CC, Lin YS et al (2010) Nuclear export and import of human hepatitis B virus capsid protein and particles. PLoS Pathog 6(10):e1001162
79. Schmitz A, Schwarz A, Foss M, Zhou L, Rabe B, Hoellenriegel J et al (2010) Nucleoporin 153 arrests the nuclear import of hepatitis B virus capsids in the nuclear basket. PLoS Pathog 6(1):e1000741
80. Rabe B, Delaleau M, Bischof A, Foss M, Sominskaya I, Pumpens P et al (2009) Nuclear entry of hepatitis B virus capsids involves disintegration to protein dimers followed by nuclear reassociation to capsids. PLoS Pathog 5(8):e1000563
81. Michael N (2015) HBV cccDNA: viral persistence reservoir and key obstacle for a cure of chronic hepatitis B. Gut 64(12):1972–1984
82. Schreiner S, Nassal M (2017) A role for the host DNA damage response in hepatitis B virus cccDNA formation-and beyond? Viruses 9(5):125
83. Guo JT, Guo H (2015) Metabolism and function of hepatitis B virus cccDNA: implications for the development of cccDNA-targeting antiviral therapeutics. Antivir Res 122:91–100
84. Cui X, McAllister R, Boregowda R, Sohn JA, Cortes Ledesma F, Caldecott KW (2015) Does Tyrosyl DNA Phosphodiesterase-2 play a role in hepatitis B virus genome repair? PLoS One 10(6):e0128401
85. Jones SA, Boregowda R, Spratt TE, Hu J (2012) In vitro epsilon RNA-dependent protein priming activity of human hepatitis B virus polymerase. J Virol 86(9):5134–5150
86. Jones SA, Hu J (2013) Protein-primed terminal transferase activity of hepatitis B virus polymerase. J Virol 87(5):2563–2576
87. Königer C, Wingert I, Marsmann M, Rösler C, Beck J, Nassal M (2014) Involvement of the host DNA-repair enzyme TDP2 in formation of the covalently closed circular DNA persistence reservoir of hepatitis B viruses. Proc Natl Acad Sci U S A 111(40):E4244–E4253
88. Gao W, Hu J (2007) Formation of hepatitis B virus covalently closed circular DNA: removal of genome-linked protein. J Virol 81(12):6164–6174
89. Seeger C, Mason WS (2015) Molecular biology of hepatitis B virus infection. Virology 479–480:672–686
90. Kitamura K, Que L, Shimadu M, Koura M, Ishihara Y, Wakae K et al (2018) Flap endonuclease 1 is involved in cccDNA formation in the hepatitis B virus. PLoS Pathog 14(6):e1007124
91. Hantz O, Parent R, Durantel D, Gripon P, Guguen-Guillouzo C, Zoulim F (2009) Persistence of the hepatitis B virus covalently closed circular DNA in HepaRG human hepatocyte-like cells. J Gen Virol 90(Pt 1):127–135
92. Qi Y, Gao Z, Xu G, Peng B, Liu C, Yan H et al (2016) DNA polymerase κ is a key cellular factor for the formation of covalently closed circular DNA of hepatitis B virus. PLoS Pathog 12(10):e1005893
93. Long Q, Yan R, Hu J, Cai D, Mitra B, Kim ES et al (2017) The role of host DNA ligases in hepadnavirus covalently closed circular DNA formation. PLoS Pathog 13(12):e1006784
94. Guo H, Xu C, Zhou T, Block TM, Guo JT (2012) Characterization of the host factors required for hepadnavirus covalently closed circular (ccc) DNA formation. PLoS One 7(8):e43270

95. Chang HHY, Pannunzio NR, Adachi N, Lieber MR (2017) Non-homologous DNA end joining and alternative pathways to double-strand break repair. Nat Rev Mol Cell Biol 18(8):495–506
96. Newbold JE, Xin H, Tencza M, Sherman G, Dean J, Bowden S et al (1995) The covalently closed duplex form of the hepadnavirus genome exists in situ as a heterogeneous population of viral minichromosomes. J Virol 69(6):3350–3357
97. Bock CT, Schwinn S, Locarnini S, Fyfe J, Manns MP, Trautwein C et al (2001) Structural organization of the hepatitis B virus minichromosome. J Mol Biol 307(1):183–196
98. Hong X, Kim ES, Guo H (2017) Epigenetic regulation of hepatitis B virus covalently closed circular DNA: implications for epigenetic therapy against chronic hepatitis B. Hepatology 66(6):2066–2077
99. Nassal M, Schaller H (1993) Hepatitis B virus replication. Trends Microbiol 1(6):221–228
100. Kitamura K, Que L, Shimadu M, Koura M, Ishihara Y, Wakae K et al (2018) Flap endonuclease 1 is involved in cccDNA formation in the hepatitis B virus. PLoS Pathog 14(6):e1007124
101. Hu J, Cheng J, Tang L, Hu Z, Luo Y, Li Y et al (2018) Virological basis for the cure of chronic hepatitis B. ACS Infect Dis 5(5):659–674
102. Kramvis A, Kew MC (1999) The core promoter of hepatitis B virus. J Viral Hepat 6(6):415–427
103. Kairat A, Beerheide W, Zhou G, Tang ZY, Edler L, Schröder CH (1999) Truncated hepatitis B virus RNA in human hepatocellular carcinoma: its representation in patients with advancing age. Intervirology 42(4):228–237
104. Lu F, Wang J, Chen X, Xu D, Xia N (2017) Potential use of serum HBV RNA in antiviral therapy for chronic hepatitis B in the era of nucleos(t)ide analogs. Front Med 11(4):502–508
105. Hiraga M, Nishizono A, Mifune K, Esumi M, Shikata T (1994) Analysis of upstream region of hepatitis B virus core gene using in vitro transcription system. J Med Virol 43(4):404–411
106. Yu X, Mertz JE (1996) Promoters for synthesis of the pre-C and pregenomic mRNAs of human hepatitis B virus are genetically distinct and differentially regulated. J Virol 70(12):8719–8726
107. Yuh CH, Chang YL, Ting LP (1992) Transcriptional regulation of precore and pregenomic RNAs of hepatitis B virus. J Virol 66(7):4073–4084
108. Guo W, Chen M, Yen TS, Ou JH (1993) Hepatocyte-specific expression of the hepatitis B virus core promoter depends on both positive and negative regulation. Mol Cell Biol 13(1):443–448
109. Gerlach KK, Schloemer RH (1992) Hepatitis B virus C gene promoter is under negative regulation. Virology 189(1):59–66
110. Will H, Reiser W, Weimer T, Pfaff E, Büscher M, Sprengel R et al (1987) Replication strategy of human hepatitis B virus. J Virol 61(3):904–911
111. Caselmann WH, Koshy R (1998) Transactivators of HBV, signal transduction and tumorigenesis Caselmann. In: Caselmann W, Koshy R (eds) Hepatitis b virus: molecular mechanisms in disease and novel strategies for therapy, vol 1. Imperial College Press, London, pp 161–181
112. Sommer G, Heise T (2008) Posttranscriptional control of HBV gene expression. Front Biosci 13:5533–5547
113. Black DL (2003) Mechanisms of alternative pre-messenger RNA splicing. Annu Rev Biochem 72:291–336
114. Su TS, Lai CJ, Huang JL, Lin LH, Yauk YK, Chang CM et al (1989) Hepatitis B virus transcript produced by RNA splicing. J Virol 63(9):4011–4018
115. Terré S, Petit MA, Bréchot C (1991) Defective hepatitis B virus particles are generated by packaging and reverse transcription of spliced viral RNAs in vivo. J Virol 65(10):5539–5543
116. Bayliss J, Lim L, Thompson AJ, Desmond P, Angus P, Locarnini S et al (2013) Hepatitis B virus splicing is enhanced prior to development of hepatocellular carcinoma. J Hepatol 59:1022–1028
117. Redelsperger F, Lekbaby B, Mandouri Y, Giang E, Duriez M, Desire N et al (2012) Production of hepatitis B defective particles is dependent on liver status. Virology 431:21–28

118. Soussan P, Pol J, Garreau F, Schneider V, Le Pendeven C, Nalpas B et al (2008) Expression of defective hepatitis B virus particles derived from singly spliced RNA is related to liver disease. J Infect Dis 198(2):218–225
119. Duriez M, Mandouri Y, Lekbaby B, Wang H, Schnuriger A, Redelsperger F et al (2017) Alternative splicing of hepatitis B virus: a novel virus/host interaction altering liver immunity. J Hepatol 67:687–699
120. Soussan P, Garreau F, Zylberberg H, Ferray C, Brechot C, Kremsdorf D (2000) In vivo expression of a new hepatitis B virus protein encoded by a spliced RNA. J Clin Invest 105:55–60
121. Bayard F, Godon O, Nalpas B, Costentin C, Zhu R, Soussan P et al (2012) T-cell responses to hepatitis B splice-generated protein of hepatitis B virus and inflammatory cytokines/chemokines in chronic hepatitis B patients. ANRS study: HB EP 02 HBSP-FIBRO. J Viral Hepat 19:872–880
122. Chen JY, Chen WN, Jiao BY, Lin WS, Wu YL, Liu LL et al (2014) Hepatitis B spliced protein (HBSP) promotes the carcinogenic effects of benzo [alpha] pyrene by interacting with microsomal epoxide hydrolase and enhancing its hydrolysis activity. BMC Cancer 14:282
123. Pol JG, Lekbaby B, Redelsperger F, Klamer S, Mandouri Y, Ahodantin J et al (2015) Alternative splicing-regulated protein of hepatitis B virus hacks the TNF-alpha-stimulated signaling pathways and limits the extent of liver inflammation. FASEB J 29:1879–1889
124. Bruss V, Gerlich WH (1988) Formation of transmembraneous hepatitis B e-antigen by cotranslational in vitro processing of the viral precore protein. Virology 163:268–275
125. Bruss V (2007) Hepatitis B virus morphogenesis. World J Gastroenterol 13:65–73
126. Chai N, Chang HE, Nicolas E, Han Z, Jarnik M, Taylor J (2008) Properties of subviral particles of hepatitis B virus. J Virol 82:7812–7817
127. Cornberg M, Wong VW, Locarnini S, Brunetto M, Janssen HLA, Chan HL (2017) The role of quantitative hepatitis B surface antigen revisited. J Hepatol 66:398–411
128. Glebe D, Urban S (2007) Viral and cellular determinants involved in hepadnaviral entry. World J Gastroenterol 13:22–38
129. Gudima S, He Y, Meier A, Chang J, Chen R, Jarnik M et al (2007) Assembly of hepatitis delta virus: particle characterization, including the ability to infect primary human hepatocytes. J Virol 81:3608–3617
130. Heermann KH, Goldmann U, Schwartz W, Seyffarth T, Baumgarten H, Gerlich WH (1984) Large surface proteins of hepatitis B virus containing the pre-s sequence. J Virol 52:396–402
131. Huovila AP, Eder AM, Fuller SD (1992) Hepatitis B surface antigen assembles in a post-ER, pre-Golgi compartment. J Cell Biol 118:1305–1320
132. Jiang B, Himmelsbach K, Ren H, Boller K, Hildt E (2015) Subviral hepatitis B virus filaments, like infectious viral particles, are released via multivesicular bodies. J Virol 90:3330–3341
133. Lok AS, Zoulim F, Dusheiko G, Ghany MG (2017) Hepatitis B cure: from discovery to regulatory approval. J Hepatol 67:847–861
134. Lok AS, Zoulim F, Dusheiko G, Ghany MG (2017) Hepatitis B cure: from discovery to regulatory approval. Hepatology 66:1296–1313
135. Murakami S (2001) Hepatitis B virus X protein: a multifunctional viral regulator. J Gastroenterol 36:651–660
136. Murakami S (1999) Hepatitis B virus X protein: structure, function and biology. Intervirology 42:81–99
137. Beck J, Vogel M, Nassal M (2002) dNTP versus NTP discrimination by phenylalanine 451 in duck hepatitis B virus P protein indicates a common structure of the dNTP-binding pocket with other reverse transcriptases. Nucleic Acids Res 30(7):1679–1687
138. Tavis JE, Massey B, Gong Y (1998) The duck hepatitis B virus polymerase is activated by its RNA packaging signal, epsilon. J Virol 72(7):5789–5796
139. Tavis JE, Ganem D (1996) Evidence for activation of the hepatitis B virus polymerase by binding of its RNA template. J Virol 70(9):5741–5750

140. Junker-Niepmann M, Bartenschlager R, Schaller H (1990) A short cis-acting sequence is required for hepatitis B virus pregenome encapsidation and sufficient for packaging of foreign RNA. EMBO J 9(10):3389–3396
141. Kim HY, Park GS, Kim EG, Kang SH, Shin HJ, Park S et al (2004) Oligomer synthesis by priming deficient polymerase in hepatitis B virus core particle. Virology 322(1):22–30
142. Hu J, Toft DO, Seeger C (1997) Hepadnavirus assembly and reverse transcription require a multi-component chaperone complex which is incorporated into nucleocapsids. EMBO J 16(1):59–68
143. Hu J, Seeger C (1996) Hsp90 is required for the activity of a hepatitis B virus reverse transcriptase. Proc Natl Acad Sci U S A 93(3):1060–1064
144. Patel N, White SJ, Thompson RF, Bingham R, Weiss EU, Maskell DP et al (2017) HBV RNA pre-genome encodes specific motifs that mediate interactions with the viral core protein that promote nucleocapsid assembly. Nat Microbiol 2:17098
145. Wang GH, Seeger C (1992) The reverse transcriptase of hepatitis B virus acts as a protein primer for viral DNA synthesis. Cell 71(4):663–670
146. Lanford RE, Notvall L, Lee H, Beames B (1997) Transcomplementation of nucleotide priming and reverse transcription between independently expressed TP and RT domains of the hepatitis B virus reverse transcriptase. J Virol 71(4):2996–3004
147. Weber M, Bronsema V, Bartos H, Bosserhoff A, Bartenschlager R, Schaller H (1994) Hepadnavirus P protein utilizes a tyrosine residue in the TP domain to prime reverse transcription. J Virol 68(5):2994–2999
148. Zoulim F, Seeger C (1994) Reverse transcription in hepatitis B viruses is primed by a tyrosine residue of the polymerase. J Virol 68(1):6–13
149. Tavis JE, Ganem D (1995) RNA sequences controlling the initiation and transfer of duck hepatitis B virus minus-strand DNA. J Virol 69(7):4283–4291
150. Tavis JE, Perri S, Ganem D (1994) Hepadnavirus reverse transcription initiates within the stem-loop of the RNA packaging signal and employs a novel strand transfer. J Virol 68(6):3536–3543
151. Wang GH, Seeger C (1993) Novel mechanism for reverse transcription in hepatitis B viruses. J Virol 67(11):6507–6512
152. Abraham TM, Loeb DD (2006) Base pairing between the 5′ half of epsilon and a cis-acting sequence, phi, makes a contribution to the synthesis of minus-strand DNA for human hepatitis B virus. J Virol 80(9):4380–4387
153. Tang H, Mclachlan A (2002) A pregenomic RNA sequence adjacent to DR1 and complementary to epsilon influences hepatitis B virus replication efficiency. Virology 303(1):199–210
154. Abraham TM, Loeb DD (2007) The topology of hepatitis B virus pregenomic RNA promotes its replication. J Virol 81(21):11577–11584
155. Haines KM, Loeb DD (2007) The sequence of the RNA primer and the DNA template influence the initiation of plus-strand DNA synthesis in hepatitis B virus. J Mol Biol 370(3):471–480
156. Loeb DD, Hirsch RC, Ganem D (1991) Sequence-independent RNA cleavages generate the primers for plus strand DNA synthesis in hepatitis B viruses: implications for other reverse transcribing elements. EMBO J 10(11):3533–3540
157. Summers J, Mason WS (1982) Replication of the genome of a hepatitis B--like virus by reverse transcription of an RNA intermediate. Cell 29(2):403–415
158. Seeger C, Ganem D, Varmus HE (1986) Biochemical and genetic evidence for the hepatitis B virus replication strategy. Science 232(4749):477–484
159. Lien JM, Aldrich CE, Mason WS (1986) Evidence that a capped oligoribonucleotide is the primer for duck hepatitis B virus plus-strand DNA synthesis. J Virol 57(1):229–236
160. Habig JW, Loeb DD (2006) Sequence identity of the direct repeats, DR1 and DR2, contributes to the discrimination between primer translocation and in situ priming during replication of the duck hepatitis B virus. J Mol Biol 364(1):32–43

161. Yang W, Summers J (1999) Integration of hepadnavirus DNA in infected liver: evidence for a linear precursor. J Virol 73(12):9710–9717
162. Staprans S, Loeb DD, Ganem D (1991) Mutations affecting hepadnavirus plus-strand DNA synthesis dissociate primer cleavage from translocation and reveal the origin of linear viral DNA. J Virol 65(3):1255–1262
163. Lewellyn EB, Loeb DD (2007) Base pairing between cis-acting sequences contributes to template switching during plus-strand DNA synthesis in human hepatitis B virus. J Virol 81(12):6207–6215
164. Liu N, Tian R, Loeb DD (2003) Base pairing among three cis-acting sequences contributes to template switching during hepadnavirus reverse transcription. Proc Natl Acad Sci U S A 100(4):1984–1989
165. Tuttleman JS, Pourcel C, Summers J (1986) Formation of the pool of covalently closed circular viral DNA in hepadnavirus-infected cells. Cell 47(3):451–460
166. Wu TT, Coates L, Aldrich CE, Summers J, Mason WS (1990) In hepatocytes infected with duck hepatitis B virus, the template for viral RNA synthesis is amplified by an intracellular pathway. Virology 175(1):255–261
167. Block TM, Guo H, Guo JT (2007) Molecular virology of hepatitis B virus for clinicians. Clin Liver Dis 11(4):685–706, vii
168. Bruss V, Ganem D (1991) The role of envelope proteins in hepatitis B virus assembly. Proc Natl Acad Sci U S A 88:1059–1063
169. Fernholz D, Stemler M, Brunetto M, Bonino F, Will H (1991) Replicating and virion secreting hepatitis B mutant virus unable to produce preS2 protein. J Hepatol 13:S102–S104
170. Jenna S, Sureau C (1998) Effect of mutations in the small envelope protein of hepatitis B virus on assembly and secretion of hepatitis delta virus. Virology 251:176–186
171. Tan WS, Dyson MR, Murray K (1999) Two distinct segments of the hepatitis B virus surface antigen contribute synergistically to its association with the viral core particles. J Mol Biol 286:797–808
172. Löffler-Mary H, Dumortier J, Klentsch-Zimmer C, Prange R (2000) Hepatitis B virus assembly is sensitive to changes in the cytosolic S loop of the envelope proteins. Virology 270:358–367
173. Wang J, Sheng Q, Ding Y, Chen R, Sun X, Chen X et al (2017) HBV RNA virion-like particles produced under nucleos(t)ide analogues treatment are mainly replication-deficient. J Hepatol. pii: S0168-8278(17)32413-3
174. Lam AM, Ren S, Espiritu C, Kelly M, Lau V, Zheng L et al (2017) Hepatitis B virus capsid assembly modulators, but not nucleoside analogs, inhibit the production of extracellular pregenomic RNA and spliced RNA variants. Antimicrob Agents Chemother 61(8):pii: e00680-17
175. DiMattia MA, Watts NR, Stahl SJ, Grimes JM, Steven AC, Stuart DI et al (2013) Antigenic switching of hepatitis B virus by alternative dimerization of the capsid protein. Structure 21(1):133–142
176. Ning X, Luckenbaugh L, Liu K, Bruss V, Sureau C, Hu J (2018) Common and distinct capsid and surface protein requirements for secretion of complete and genome-free hepatitis B Virions. J Virol 92:pii: e00272-18
177. Bardens A, Döring T, Stieler J, Prange R (2011) Alix regulates egress of hepatitis B virus naked capsid particles in an ESCRT-independent manner. Cell Microbiol 13:602–619
178. Döring T, Prange R (2015) Rab33B and its autophagic Atg5/12/16L1 effector assist in hepatitis B virus naked capsid formation and release. Cell Microbiol 17:747–764
179. Bai L, Zhang X, Kozlowski M, Li W, Wu M, Liu J et al (2018) Extracellular hepatitis B virus RNAs are heterogeneous in length and circulate as capsid-antibody complexes in addition to Virions in chronic hepatitis B patients. J Virol 27:92(24)

180. Summers J, O'Connell A, Millman I (1975) Genome of hepatitis B virus: restriction enzyme cleavage and structure of DNA extracted from Dane particles. Proc Natl Acad Sci U S A 72(11):4597–4601
181. Sureau C, Salisse J (2013) A conformational heparan sulfate binding site essential to infectivity overlaps with the conserved hepatitis B virus a-determinant. Hepatology 57(3):985–994
182. Yang HC, Kao JH (2014) Persistence of hepatitis B virus covalently closed circular DNA in hepatocytes: molecular mechanisms and clinical significance. Emerg Microbes Infect 3(9):e64
183. Zhang YY, Zhang BH, Theele D, Litwin S, Toll E, Summers J (2003) Single-cell analysis of covalently closed circular DNA copy numbers in a hepadnavirus-infected liver. Proc Natl Acad Sci U S A 100(21):12372–12377
184. Yang W, Summers J (1995) Illegitimate replication of linear hepadnavirus DNA through nonhomologous recombination. J Virol 69(7):4029–4036
185. Bill CA, Summers J (2004) Genomic DNA double-strand breaks are targets for hepadnaviral DNA integration. Proc Natl Acad Sci U S A 101(30):11135–11140
186. Li X, Zhang J, Yang Z, Kang J, Jiang S, Zhang T et al (2014) The function of targeted host genes determines the oncogenicity of HBV integration in hepatocellular carcinoma. J Hepatol 60(5):975–984
187. Wang HP, Rogler CE (1991) Topoisomerase I-mediated integration of hepadnavirus DNA in vitro. J Virol 65(5):2381–2392
188. Mason WS, Gill US, Litwin S, Zhou Y, Peri S, Pop O et al (2016) HBV DNA integration and clonal hepatocyte expansion in chronic hepatitis B patients considered immune tolerant. Gastroenterology 151(5):986–998
189. Rivkina MB, Lunin VG, Mahov AM, Tikchonenko TI, Kukain RA (1988) Nucleotide sequence of integrated hepatitis B virus DNA and human flanking regions in the genome of the PLC/PRF/5 cell line. Gene 64(2):285–296
190. Hu B, Wang R, Fu J, Su M, Du M, Liu Y et al (2018) Integration of hepatitis B virus S gene impacts on hepatitis B surface antigen levels in patients with antiviral therapy. J Gastroenterol Hepatol 33(7):1389–1396
191. Wooddell CI, Yuen MF, Chan HL, Gish RG, Locarnini SA, Chavez D et al (2017) RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg. Sci Transl Med 9(409):pii: eaan0241