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Hepatitis B Virus in Human Diseases

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Hepatitis B Virus in Human Diseases

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Foreword

The publication of "Hepatitis B virus in Human Diseases" coincides with the fiftieth anniversary of the discovery of the virus [1]. This is a good time, therefore, to reflect on where we have come from, and are going.

We now know that Hepatitis B virus (HBV) is a small DNA virus that has chronically infected hundreds of millions of people worldwide and is responsible for nearly a million liver disease-related deaths a year [2, 3]. However, it is worth noting that the discovery of hepatitis B was not an obvious one, but was made in a very exciting time. Although science progressed with great speed in the 1940s, 1950s, and early 1960s, most of this progress was in molecular biology, as compared with medical science. For example, and to put the period in context, DNA was identified as the information molecule in all cells [4] and its structure of DNA was solved by Watson and Crick [5]. The genetic code was translated by Nirenberg and Matthaei [6] and the genetic regulation of protein synthesis was discovered by Jacob and Monod [7]. These fundamental discoveries were made possible by rigorous application of the scientific method. If medical science progress was slower, it may have been because it focused on detailed descriptions of disease rather than applications of the scientific method to discover etiology. Discovery of HBV came after a series of clever insights standing on what could have been taken as unrelated discoveries and observations.

Baruch S Blumberg, who received the 1976 Nobel Prize in Medicine or Physiology for his role in the discovery of hepatitis B and chronic viral diseases, had studied biochemistry with Alexander Ogston at Oxford University in the late 1950s. There he learned that British research used a "scientific" method to investigate medical and biological problems. His work began with the study of protein polymorphisms in peripheral blood. Initially, Blumberg and Allison determined if people who received transfusions made antibodies to antigens on polymorphic blood proteins [8]. Later Blumberg and Alter continued to test this hypothesis using serum from multiply transfused patients, to identify more polymorphic proteins. One such protein, they called "Australia" antigen, named for the location of individual in whose blood it was found [1]. Careful testing of sera from patients with a variety of diseases eventually led to finding the association of Australia antigen with

one type of viral hepatitis, once called "serum hepatitis," and is now called hepatitis B. Surprisingly the Australian antigen was located on the surface of the virus itself and is now called HBsAg. The complicated natural history of hepatitis B disease in people made the discovery of its etiology all the more remarkable. Blumberg viewed it as a vindication of both non-goal-oriented research and the application of the scientific method to human disease.

Realization that Australia Antigen, originally recognized only by precipitin lines in Ouchterlony gel plates [1], was associated with hepatitis [9] and the hepatitis B virus envelope protein [10, 11] led to development of an assay to screen donor bloods and to invention of an effective vaccine [12]. The screening assay rapidly led to clearance of the blood supply of virally contaminated blood. Harvey Alter led the call to test all blood to be used for transfusions for the presence of "Australia" antigen based on these early observations and thus affected an important translation of basic scientific findings into an important clinical application in an unprecedentedly brief time.

The first HBV vaccine was produced from viral antigen derived from chemically inactivated virus, isolated from the blood of infected carriers [12], and approved for use by the US FDA in 1981. This was replaced in the 1990s with vaccines made from HBV recombinant envelope protein, isolated from yeast or mammalian cell culture (CHO cells), thus avoiding the concerns surrounding use of human HBV carrier blood [13]. These vaccines have been effective in interrupting perinatal transmission (which is a form of "post-exposure" protection), as well as other "horizontal" transmissions of the virus [14]. The effectiveness of these vaccines, all of which are formulations of purified HBsAg proteins (rather than live, replicating virus), is, and in itself, as surprising as it is important and instructive. Indeed, the discovery of HBV and development and use of an effective vaccine is one of the great accomplishments in medical and public health of the last century.

Realization that HBV is associated with hepatocellular carcinoma (HCC) must also be considered a great scientific accomplishment [15–17]. The demonstration that vaccination against HBV resulted in reducing the incidence of HCC both showed the public health benefit of vaccination [18] and providing final, definitive evidence of a cause and effect relationship between the virus and the cancer.

However, how HBV causes HCC remains elusive. The mechanism of oncogenesis almost certainly involves the necro-inflammatory pathogenesis associated with most chronic hepatitis B, but there is no specific viral oncogene, and replication of the virus in hepatocytes does not usually kill the infected cell [14]. Indeed, how the virus replicates has also generated some surprises. For example, the discovery that, although HBV is a DNA virus, it replicates through an RNA intermediate, and uses a virus-specified reverse transcriptase, is one of the major non-obvious findings in virology of the last part of the twentieth century [19]. It is also the molecular basis for the action for the small molecule direct acting hepatitis B antivirals [20] that are changing the natural history of the disease.

Parenthetically, one of the more dramatic demonstrations of how intervention with only polymerase inhibitors (in this case, lamivudine) can affect the natural history of chronic hepatitis was reported by this book's co-editor, Dr. Liaw [21].

The discovery of hepatitis D, a "viroid" that requires hepatitis B co-infection for it to complete its replication cycle, and exacerbates chronic hepatitis B, must also be considered to be another enormously significant medical and scientific finding that is a part of the hepatitis B story [22]. Hepatitis D continues to be a major, although often overlooked health threat.

Hepatitis B remains a vital topic for study: with somewhere between 250 and 350 million people chronically infected with the virus, and as many as 25 % may die from liver disease (liver cirrhosis or hepatocellular carcinoma), without beneficial intervention [3].

Thus, chronic hepatitis B (but not hepatitis D) is now treatable. The polymerase inhibitors, and interferons, are enabling achievement of viral suppression and improvement of liver function. Indeed, discussion about these advances is found in this book. However, a medical cure for hepatitis B is not yet available, and continued research is still needed to achieve a cure of the infection and prevent HCC, its most deadly outcome. We went 25 years from the discovery of hepatitis C to a definitive cure. The part of the story that tells of a cure for hepatitis B, even after 50 years, still needs to be written.

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Preface

The discovery of Australia antigen 50 years ago, with subsequent link to hepatitis B virus (HBV), has opened up a golden era of hepatitis research. Tremendous advances in both basic and clinical aspects of HBV have been achieved in the past five decades. However, HBV remains a major public health problem worldwide and is still the leading cause of hepatocellular carcinoma, one of the most deadly cancers. The field of HBV infections continues to evolve, allowing maximal benefit for patients. This is manifest in higher response rate to antiviral therapy and prevention of liver disease complications in infected patients, and better prophylaxis for non-infected ones. The HBV research field is currently living a new momentum with major discoveries on its life cycle for instance with the discovery of the receptor for virus entry or the identification of key cellular enzymes involved in the formation of viral cccDNA, and research efforts for the identification of novel treatment targets towards a real cure of the infection.

To mark and celebrate the fiftieth anniversary of HBV discovery, world renowned HBV experts have reviewed the development/advancement in their respective fields, as compiled in this book. Thanks to the contribution of these experts, this textbook has provided a comprehensive, state-of-the art review of this field. The different chapters review new data about basic and translational science including the viral life cycle, the immunopathogenesis of virus-induced chronic hepatitis, the mechanism of virus-induced liver cancer, and their potential applications for the clinical management of patients. The book also provides a comprehensive review of the clinical aspects of this chronic viral infection with important chapters on the global epidemiology, the natural history of the disease, and the management of special patient populations. Important chapters on the management of antiviral therapy and the recent international guidelines for the treatment of hepatitis B should help clinicians in their daily decisions when treating patients. Finally, the book reviews the current state of the art regarding immunoprophylaxis to prevent the spread of the virus and its major clinical consequences. The new advances and perspectives in the development of improved antiviral treatments are discussed as they may pave the way towards novel therapeutic concepts which, together with mass vaccination programs, should significantly impact the disease burden hopefully in a near future.

The content of this book may have shed light on and may help in the development of new viewpoints and approaches in hepatitis B research and clinical hepatology. Hopefully this book should serve as a valuable resource for students, clinicians, and researchers with an interest in hepatitis B. In this regard, we would like to express our deep appreciation to the authors of this book. We would also like to acknowledge our collaborators who helped in the reviewing of the chapters:

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Chapter 1
Hepatitis B Virus Virology and Replication

Jianming Hu

The Virus and Classification

Discovered 50 years ago as an antigenic "polymorphism" in an Australian aborigine—the "Australia antigen" [1], the hepatitis B virus (HBV) remains today a major global pathogen that causes acute and chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [2]. Owing to its unique replication strategy, as will be detailed below, HBV is classified into its own family, *Hepadnaviridae* [3], along with related animal viruses. The latter include the woodchuck hepatitis virus [4], particularly useful as a model for studying HBV pathogenesis, and the duck hepatitis B virus (DHBV) [5], particularly useful for studies on viral replication. HBV and DHBV represent, respectively, the type member of the two separate genera within the family, the mammalian and avian hepadnaviruses that infect a number of mammalian and avian species. All hepadnaviruses share strict species and tissue tropism—mostly restricted to hepatocytes in their respective hosts, and a unique life cycle—replicating a double-stranded (DS) DNA genome via an RNA intermediate and are thus sometimes called retroid viruses or para-retroviruses [3, 6].

The Virions and Subviral Particles

The complete HBV virion is a sphere with a diameter of ca. 40–45 nm, first visualized using transmission electron microscopy (EM) (the so-called Dane particle) [7] and more recently, and with much greater detail, using cryo-EM [8, 9].

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The virion has an outer envelope, which is studded with viral envelope (surface) proteins and surrounds an icosahedral capsid that, in turn, encloses the DS DNA genome. The complete virion is extremely infectious, with one virion being able to cause productive infection in chimpanzees that, in addition to humans, are susceptible to HBV [10]. On the other hand, a huge excess (100–100,000-fold over the complete virion) of noninfectious viral particles that contain no viral genome are also produced during infection. These so-called subviral particles include the classical spheres and filaments, which are ca. 22 nm in diameter and contain only the outer envelope layer of the virion, and the recently discovered empty virions, which contain both the outer envelope and the inner capsid shell but no viral DNA or RNA [11, 12].

Viral DNA Structure and Genome Organization

All hepadnaviruses share a peculiar virion DNA structure (Fig. 1.1) [13–15]. The DNA is small (3.2 kbp for HBV and 3.0 kbp for DHBV) and is held in a circular configuration via complementarity at the 5′ ends of both DNA strands, the length of complementarity being ca. 200 nucleotides (nt) in HBV and 60 nt in DHBV. Neither of the two strands of this relaxed circular DNA (rcDNA) is covalently closed. Whereas the (−) strand, i.e., the DNA strand complementary to pgRNA, has a short (ca. 9 nt) terminal redundancy (r), the other strand, the (+) strand, is heterogeneous in length with 3′ ends terminating hundreds of nt before completion. The rcDNA is further modified by a covalently linked protein (the terminal protein or TP) [16], later shown to be part of the viral reverse transcriptase (RT) or polymerase (P) protein [17] that is used to prime (−) DNA synthesis, and a capped, 18 nt-long RNA oligomer attached to the 5′ end of (+) DNA resulting from its use as a primer to initiate (+) strand DNA synthesis [14, 15] (see section "Reverse Transcription and NC Maturation" below).

Four distinct classes of viral mRNAs, all 5′ capped and 3′ polyadenylated, are encoded by the viral DNA. The genomic PreC/C mRNA is in fact longer than the DNA template (i.e., overlength), being 3.5 kb in length. The subgenomic PreS1, PreS2/S, and X mRNAs are approximately 2.4 kb, 2.1 kb, and 0.7 kb long, respectively. All viral mRNAs share the same 3′ sequences as represented by the shortest X mRNA, since they all terminate at the single polyadenylation signal. Transcription of these four groups of mRNAs is driven by four different viral promoters, respectively, the core, PreS1, PreS2/S, and X promoters that are further regulated by two viral enhancers, enhancer I upstream and overlapping with the X promoter and enhancer II located upstream of the core promoter (Fig. 1.1). A total of seven viral proteins are produced from these mRNAs using four open reading frames (ORF) (Fig. 1.1). The PreC/C mRNAs encode the viral core or capsid (C) protein and the slightly longer PreC protein using the same ORF, and the P protein using an alternative reading frame. As will be detailed below, the shortest of these genomic RNAs also serves as the template for reverse transcription to reproduce rcDNA

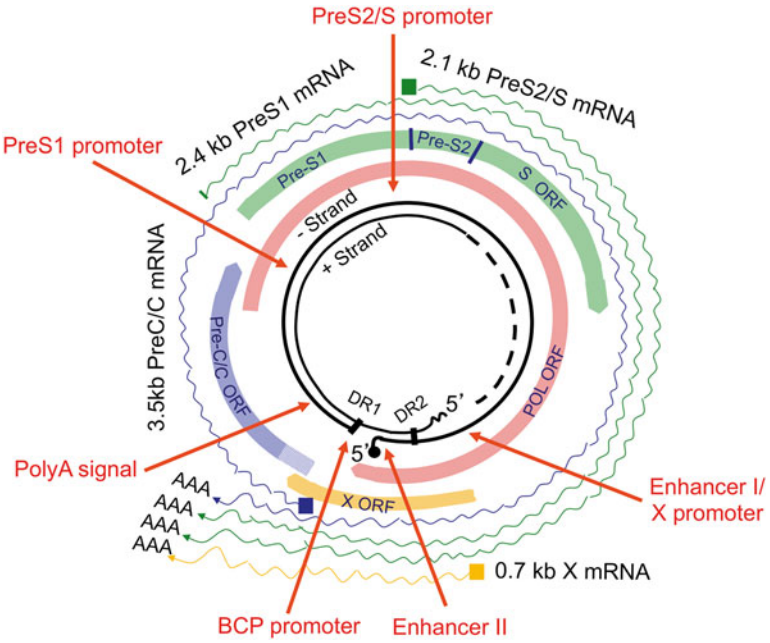

Fig. 1.1 HBV DNA structure and genome organization. The *inner circle* represents the virion rcDNA, and the *dashes* represent the region of the (+) strand DNA that is yet to be synthesized. The 5′ ends of the (−) and (+) strands are indicated. The *small*, *filled circle* represents the P protein covalently attached to the 5′ end of the (−) strand, and the *short wavy line*, the capped RNA oligomer attached to the 5′ end of the (+) strand. The *vertical bars* on rcDNA denote the direct repeats 1 and 2 (DR1 and DR2). The short terminal redundancy (r) on the (−) strand is denoted by the flap attached to the P protein (for clarity, it is not labeled in the figure). The promoter and enhancer positions are indicated. The *middle circle* of *shaded boxes* represent the four open reading frames (ORFs) corresponding to the precore/core, X, polymerase, and surface proteins, with their C-terminal ends denoted by the *arrows*. The *outer circle of wavy lines* represent the viral RNAs. *Filled squares* at one end of the mRNAs denotes heterogeneous 5′ ends (PreC/C, PreS2/S, and X mRNAs), the *thin vertical line* represents the precise 5′ end of the PreS1 mRNA. The *arrows* at the other end of the lines denote the 3′ ends of the mRNAs with polyA tails (AAA). *BCP* basal core promoter, *Pol* polymerase, *PolyA* polyadenylation

during replication and is thus termed pregenomic RNA or pgRNA. The PreS1, PreS2/S, and X mRNAs encode, respectively, the large (L) envelope protein, and the middle (M) and small (S) envelope proteins, and the X protein. All three envelope (or surface) proteins are encoded within a single ORF, which is entirely embedded within the alternative P ORF (Fig. 1.1). The P gene also overlaps with the 3′ end of the C gene and 5′ end of the X gene at its 5′ and 3′ ends respectively. In addition, all transcriptional regulatory elements including the promoters, enhancers, and the polyadenylation signal overlap with the protein-coding sequences. The genomic organization of hepadnaviruses is thus characterized by extreme economy.

Structure and Functions of Viral Proteins

The Envelope Proteins

Of the three HBV envelope proteins, the smallest, S, is 226 residues long and is the most abundant. M contains a N-terminal extension, relative to S, called the PreS2 region, which is 55 residues long (Fig. 1.1). L is the longest and contains yet another N-terminal extension called the PreS1 region, which is 108 (or 119 depending on the strains) residues long. In addition to being major constituents of the virions, the envelope proteins are also secreted in large excess to the blood stream of infected people as spheres and filaments in the absence of capsids or genome, as mentioned above. Indeed, it was the abundance of these particles that allowed the discovery of HBV as the Australian antigen, i.e., hepatitis B surface antigen (HBsAg). The spheres contain mostly S and some M, and the filaments have in addition some L, which is enriched in virion particles [18]. Both L and S are required for virion secretion but M is dispensable [19]. In particular, the PreS1 region in L contains determinants required for both capsid envelopment during virion formation as well as receptor binding during entry (see below) [20]. This dual role of PreS1 is facilitated by its dynamic dual topology in the virions [21, 22]. Immediately following translation in the endoplasmic reticulum (ER) membrane, all PreS1 is located on the cytosolic side allowing it to interact with the capsids to fulfill its role in virion formation; as the virions traffic through the cellular secretory pathway, ca. 50 % of PreS1 is translocated from the interior of the virions to the exterior to allow it to bind the cell surface receptor. How this dramatic gymnastic feat is accomplished remains an enigma.

The C Protein and e Antigen

The C protein is 183 (or 185 depending on the strains) residues long. C can be divided into two structural and functional domains. The N-terminal 140 residues form the assembly domain (NTD) that is sufficient to mediate capsid assembly [23, 24]. The C-terminal domain (CTD) is dispensable for capsid assembly but plays essential roles in packaging of pgRNA into replication-competent nucleocapsids (NCs) and in reverse transcription of pgRNA to rcDNA. The C protein rapidly forms dimers, which are the building blocks for capsid assembly. Two morphological capsid isomers, with either 120 ($T=4$, the major isomer) or 90 dimers ($T=3$), are formed [25, 26]. The functional significance, if any, of this dichotomy is unknown. The arginine-rich CTD is highly basic and has nonspecific nucleic acid binding activity [27]. It also harbors multiple nuclear localization signals (NLSs) [28–30] that may be important for delivery of NCs to the nucleus (see section “Intracellular Trafficking and Uncoating” below).

Moreover, CTD is heavily phosphorylated when expressed in mammalian cells, with three major sites of phosphorylation all displaying the Ser-Pro motifs [28, 31] plus three to four additional minor sites of phosphorylation [32]. As will be described below, CTD phosphorylation plays critical roles for C functions in viral replication. As HBV does not encode any viral kinase, it has to usurp host protein kinases for C phosphorylation. A number of cellular kinases, including protein kinase C (PKC) [33], cyclin-dependent protein kinase 2 (CDK2) [34], serine-arginine protein kinase (SRPK) [35] have been reported to phosphorylate C or specifically its CTD. Among these, CDK2 has been shown to associate with and phosphorylate the CTD, in particular, its Ser-Pro sites (consistent with the known substrate specificity of CDK2 as a well-known proline-directed kinase), and is incorporated into the capsids (see below) [34, 36]. As will be detailed below, CTD phosphorylation is highly dynamic and a dramatic dephosphorylation of CTD is shown to accompany viral DNA synthesis in the DHBV NCs. The cellular phosphatase(s) responsible for C dephosphorylation remains to be identified.

The precore (PreC) protein is translated from its own mRNA (PreC mRNA), which differs from the C mRNA (pgRNA) by a 5′ extension some 30 nt long. The sequence of PreC is thus essentially the same as C, except for an additional 29 amino acids at its N-terminus [37, 38]. However, these two proteins are functionally very different; unlike C, PreC is entirely dispensable for viral replication and mutants unable to express this protein are frequently selected late during persistent infection [38]. The first 19 residues of PreC comprise a secretion signal that induces its translocation into the lumen of the ER, where the signal sequence is cleaved off by a host cell signal peptidase. The remainder of PreC undergoes further proteolytic processing (e.g., by furin) in the host cell secretory pathway to remove the highly basic CTD in C, resulting the secretion of a heterogeneous population of soluble, dimeric proteins [39, 40], defined serologically as the hepatitis B e antigen (HBeAg) (Fig. 1.2, 9c) [41]. While dispensable for viral replication, PreC/HBeAg appears to play an important role in vivo for establishing persistent infection by regulating host immune response against the related and highly immunogenic C protein [42]. Also, serum HBeAg has proven to be a useful marker to monitor viral replication as its presence tends to correlate with high levels of viral replication and its loss usually signifies a decrease in viral replication [38].

The Reverse Transcriptase

The HBV RT or P protein is a multifunctional protein that plays a central role in viral replication. P is 832 or (or 845 depending on the strains) residues long and can be divided into four separate domains, from the N-terminus: TP, the spacer, the RT domain, and the RNase H domain [43–47]. TP harbors the invariant Tyr residue essential for priming reverse transcription [48–50] (see section “Reverse

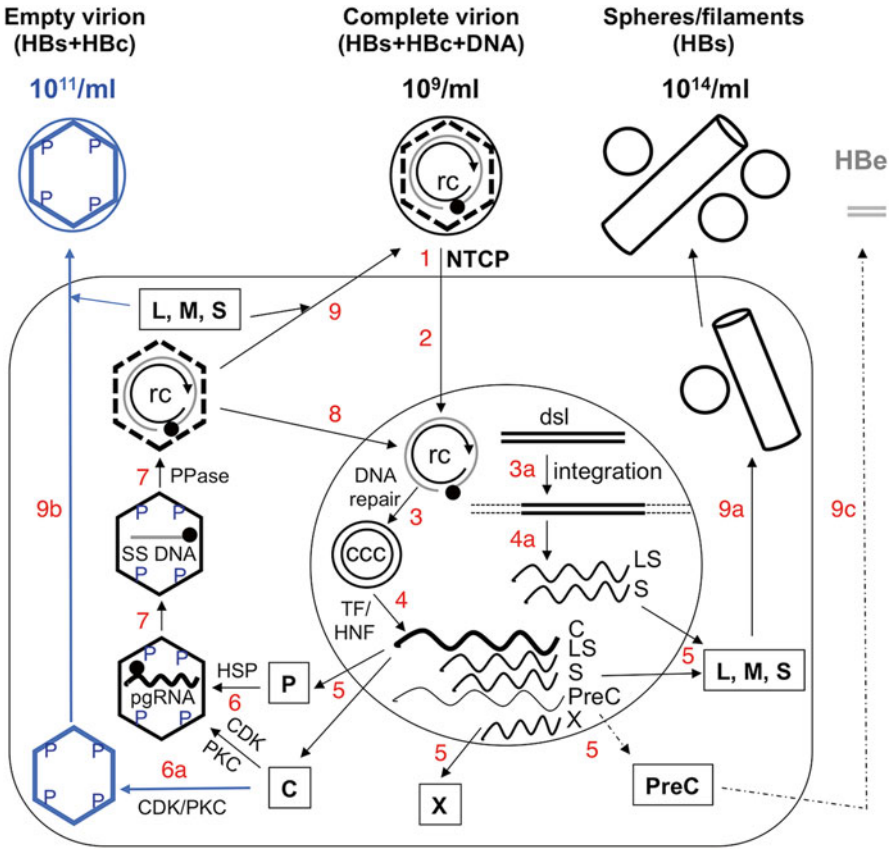

Fig. 1.2 HBV life cycle. The replication cycle of HBV is depicted schematically. (*1*) Virus binding and entry into the host cell (*large rectangle*). (*2*) Intracellular trafficking and delivery of rcDNA to the nucleus (*large circle*). (*3*) Repair of rcDNA to form cccDNA, or integration of dslDNA into host DNA (*3a*). (*4* and *4a*) Transcription to synthesize viral RNAs. (*5*) Translation to synthesize viral proteins. (*6*) Assembly of the pgRNA-containing NC, or alternatively, empty capsids (*6a*). (*7*) Reverse transcription to make the (−) strand DNA and then rcDNA. (*8*) Nuclear recycling of progeny rcDNA. (*9*) Envelopment of the rcDNA-containing NC and secretion of complete virions, or alternatively, secretion of empty virions (*9b*) or HBsAg spheres and filaments (*9a*). Processing of the PreC protein and secretion of HBeAg are depicted in (*9c*). The different viral particles outside the cell are depicted schematically with their approximate titers indicated: the complete or empty virions as *large circles* (outer envelope) with an *inner diamond shell* (capsid), with or without rcDNA inside the capsid respectively; HBsAg spheres and filaments as *small circles* and *cylinder*. Intracellular capsids are depicted as *diamonds*, with either SS [(−) strand] DNA (*straight line*), viral pgRNA (*wavy line*), or empty, and the letters "P" denoting phosphorylated residues on the immature NCs (containing SS DNA or pgRNA) or empty capsid. The *dashed lines* of the *diamond* in the rcDNA-containing mature NCs signify the destabilization of the mature NC, which is also dephosphorylated. The soluble, dimeric HBeAg is depicted as *grey double bars*. The *dashed line* and *arrow* denote the fact that HBeAg is not always secreted during viral replication. The *wavy lines* denote the viral RNAs: C, mRNA for the C and P protein (and pgRNA); S and LS, mRNAs for the S/M and L envelope proteins, respectively; PreC, mRNA for the PreC protein and following

Transcription and NC Maturation"), and together with the RT domain, are required for specific binding of pgRNA for its encapsidation into NCs. pgRNA packaging requires also the RNase H domain but none of the known enzymatic activities of P [51, 52]. The spacer region is the least conserved of the four domains and is dispensable for all known functions of P. However, its coding sequences have to be retained to encode the PreS1 region in the overlapping S ORF, which is essential for the virus as discussed above. The RT domain harbors the polymerase active site essential for DNA polymerization [44, 45], particularly the Tyr-Met-Asp-Asp motif conserved across all RT proteins including those in retroviruses and retrotransposons. The RNase H domain is responsible for degrading the pgRNA template during (−) strand DNA synthesis [44, 45, 53].

The HBV DNA polymerase activity was discovered early on, before it was realized that HBV replicates via reverse transcription, via the so-called endogenous polymerase assay [54] whereby a DNA polymerase activity in the virions was shown to carry out DNA synthesis using the endogenous virion DNA as a template. It was only a decade later that Summers and Mason made the landmark discovery that a DNA virus (i.e., DHBV) replicates through reverse transcription of an RNA intermediate [6]. However, biochemical studies on this important enzyme have proven difficult to date, and no high-resolution structures of P are yet available. As will be detailed below (section "NC Assembly"), the discovery that P requires specific host factors for its folding and functions provides at least a partial explanation to this difficulty.

The X Protein

The 154 residue-long hepatitis B X protein (HBx) is the smallest HBV protein but is arguably the least understood. There is general agreement that X is required for viral replication in vivo [55] and perhaps contributes to viral pathogenesis (for a recent review, see ref. [56]). Numerous reports have suggested a large number of functions for X in the regulation of viral and host gene expression [57], DNA damage repair [58], Ca^{2+} signaling [59], cell cycle [60], apoptosis [61], and autophagy [62, 63]. As there is no evidence that X has DNA binding activity, it is thought to affect gene expression through host protein interactions, which are probably also

Fig 1.2 (continued) processing, HBeAg. *Boxed letters* denotes the viral proteins translated from the RNAs. The *filled circle* on rcDNA denotes the P protein attached to the 5′ end of the (−) strand (*outer circle*) of rcDNA and the *arrow* denotes the 3′ end of the (+) strand (*inner circle*) of rcDNA. *ccc* cccDNA, *dsl* double stranded linear DNA, *HNF* hepatocyte nuclear factor, *HSP* heat shock protein, *PPase* phosphatase, *rc* rcDNA, *TF* transcription factor. For simplicity, the synthesis of dslDNA (the minor genomic DNA form) in the mature NC, its secretion in virions, and infection of dslDNA-containing virions are not depicted here, as are the functions of X. See text for details

important for the various other viral or cellular effects attributed to X. It remains to be clarified how the various activities attributed to X are related to each other, and how they in turn relate to viral replication and/or pathogenesis (esp., hepatocarcinogenesis). As these activities are uncovered usually using different systems and assays, which are often less than physiologically optimal due to experimental limitations, and the role of X in viral replication or pathogenesis is likely regulatory and indirect, the interpretation of the results obtained, which can be in apparent conflicts, is by no means straight-forward. Recent attempts at standardization of experimental systems and assays and the development of more physiologically relevant systems will hopefully help clarify the functions of HBx in viral replication and pathogenesis [64].

Viral Life Cycle

As a para-retrovirus, the HBV life cycle (Fig. 1.2) shares a number of similarities to conventional retroviruses, including, of course, the central role of reverse transcription. However, HBV and hepadnaviruses in general have indeed a rather unique replication strategy, which is different from the conventional retroviruses in a number of important aspects including the initiation of reverse transcription and NC assembly, genome maintenance, and virion morphogenesis.

Entry

The strong species and tissue tropism of hepadnaviruses are in part underpinned by viral entry. Until recently, the only cells in culture that are reported to support HBV infection reproducibly are primary hepatocytes from humans [65] and the small primate tupaia [66], and one human hepatoma cell line HepaRG, which requires differentiation in vitro for even the rather low infection efficiency achieved [67]. Very recently, hepatocyte-like cells differentiated from induced human pluripotent stem cells [68], and a newly established human hepatoma cell line HLCZ01 [69], are reported to support limited HBV infection.

After many false starts, the primary entry receptor for HBV was finally identified in 2012 as a hepatic bile acid transporter, sodium taurocholate cotransporting polypeptide (NTCP) (Fig. 1.2, step 1) [70]. This breakthrough allows the establishment of convenient hepatoma cell lines such as HepG2 and Huh7, which have been the mainstay for studying other aspects of HBV replication and can now support infectious entry via NTCP reconstitution. On the other hand, NTCP is insufficient to render mouse hepatocytes susceptible to HBV infection [71, 72]. This result, though disappointing, is not unexpected given previous observations that another essential, intracellular, stage in the viral life cycle, the formation of the covalently closed circular DNA (cccDNA), is also defective in mouse hepatocytes (see section "Nuclear Recycling of rcDNA and Amplification of cccDNA"). It is clear that