

RNA-protein interactions in hepadnavirus reverse transcription

Jianming Hu, Li Lin

Department of Microbiology and Immunology, The Pennsylvania State University College of Medicine, Hershey, PA 17033

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. RT-pgRNA interaction is critical for packaging of pgRNA into nucleocapsids
 - 3.1. pgRNA determinants
 - 3.1.1. ϵ RNA
 - 3.1.2. Two regions are required for DHBV pgRNA packaging
 - 3.1.3. The pgRNA cap
 - 3.2. The RT protein determinants
4. RT-pgRNA interaction is required for viral DNA synthesis
 - 4.1. Initiation of reverse transcription – protein priming
 - 4.2. Minus strand DNA elongation
5. The core protein plays an active role in pgRNA packaging and DNA synthesis
6. Specific host factors are involved in nucleocapsid assembly and reverse transcription
 - 6.1. Host chaperones are required for RT- ϵ interaction
 - 6.2. Other host factors
7. Perspectives
8. Acknowledgments
9. References

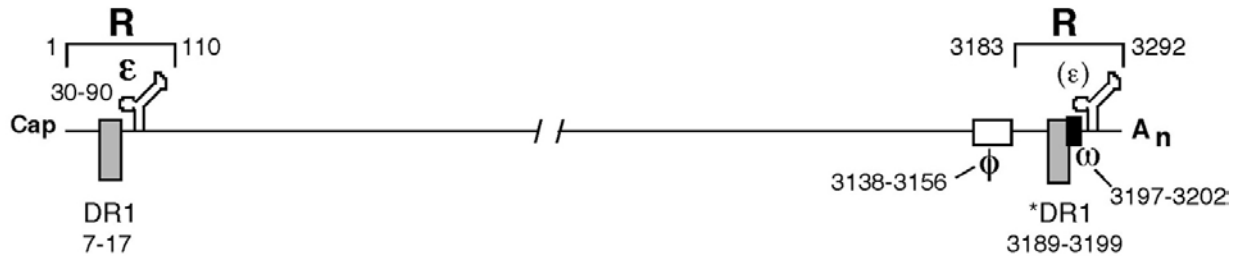
1. ABSTRACT

The small DNA genome of hepadnaviruses is replicated by reverse transcription via an RNA intermediate. This RNA “pregenome” contains important signals that control critical steps of viral replication, including RNA packaging, initiation of reverse transcription, and elongation of minus strand DNA, through specific interactions with the viral reverse transcriptase, the capsid protein, and host factors. In particular, the interaction between the viral reverse transcriptase and RNA pregenome requires a host chaperone complex composed of the heat shock protein 90 and its cochaperones.

2. INTRODUCTION

Hepadnaviruses are small DNA viruses that replicate through an RNA intermediate, and are thus called para-retroviruses (1-3). The *Hepadnaviridae* family includes the human pathogen, the hepatitis B virus (HBV), and its relatives that infect other mammalian and avian hosts (e.g., the duck HBV or DHBV). The RNA template for viral reverse transcription, the so-called pregenomic RNA (pgRNA), is transcribed in the host cell nucleus from a covalently closed circular (CCC) DNA template, which, in turn, is derived from a short (ca 3 kb), relaxed circular (RC), and partially double-stranded DNA genome present

HBV



DHBV

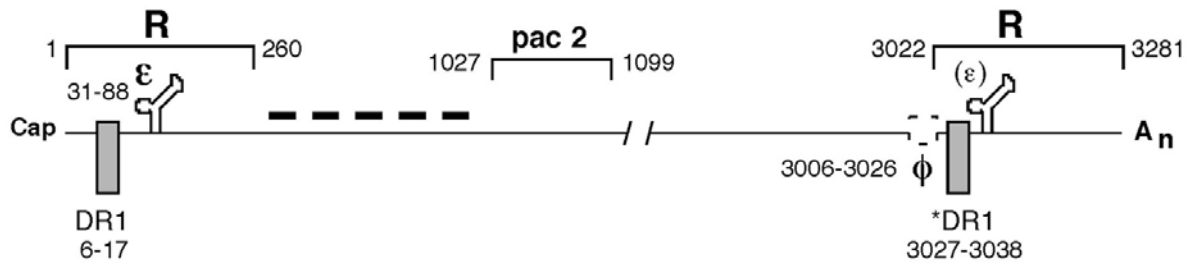


Figure 1. Summary of cis-acting signals on pgRNA of HBV and DHBV involved in RNA packaging and reverse transcription. The terminal redundancy (R) harbors the RNA packaging signal (ϵ), the DNA replication element direct repeat 1 (DR1), and the polyadenylation site. Note that the polyadenylation signal within the 5' R is not utilized and omitted for clarity. The 3' ϵ (in parenthesis) is not functional in mediating RNA packaging. The * symbol before the 3' DR1 denotes the fact that only this copy of DR1 is used as the acceptor site during minus strand transfer. The nucleotide positions (with the cap site defined as nucleotide 1) of the various signals are indicated. Pac 2 denotes a second region of pgRNA required for RNA packaging in DHBV. The two elements required for specifying the acceptor site during minus strand DNA transfer, ϕ and ω , are indicated in HBV. The ϕ element proposed for DHBV (broken box) has not been experimentally confirmed. The dash line denotes the fact that the intervening sequences between ϵ and pac 2 also contribute to pgRNA packaging in DHBV. See text for details.

in the virions. Like a typical mRNA, pgRNA carries a 5' cap and 3' poly (A) tail. It is ca 3.5 kb-long, terminally redundant (Figure 1), and is transported into the cytoplasm as an unspliced messenger RNA. Once in the cytoplasm, it first acts as a translational template for the synthesis of the viral core protein and a specialized reverse transcriptase (RT). Subsequently, pgRNA is sequestered into cytoplasmic nucleocapsid particles, where it serves its second essential function in viral replication, i.e., as the template for viral DNA synthesis.

3. RT-pgRNA INTERACTION IS CRITICAL FOR PACKAGING OF PGRNA INTO NUCLEOCAPSIDS

Upon the production of the RT protein, pgRNA is converted from an mRNA to a template for reverse transcription, through its specific incorporation into assembling nucleocapsids. In contrast to retroviruses where the RT protein is not required for the packaging of the viral genomic RNA, the hepadnavirus RT protein is absolutely essential for pgRNA packaging into nucleocapsids. Both genetic and biochemical studies demonstrate that the formation of a specific ribonucleoprotein (RNP) complex between RT and an RNA signal, termed ϵ , is critical for the encapsidation of pgRNA (as well as the RT protein) (4-6).

ϵ is located within the terminally redundant ends of pgRNA (7); however, only the 5' copy is required for RNA packaging (see Section 1.1.3 later) and, so far, no function has been attributed to the 3' copy. While ϵ is sufficient for RNA packaging in HBV, in DHBV, a second RNA signal located approximately 1,000 nucleotides downstream from ϵ is also required (Figure 1, pac 2; Section 1.1.2) (8, 9). Subsequent to RT- ϵ interaction, it is thought that 180 or 240 copies of the viral core protein, in 90 or 120 dimeric units, assemble around the RNP complex, leading to the specific incorporation of both the RT protein and pgRNA into nucleocapsids. As will be described later (Section 2.1), the interaction between ϵ and RT also plays a critical role in the initiation of reverse transcription, in addition to its essential role in nucleocapsid assembly.

Investigations of the determinants on pgRNA and the RT protein that control RNA packaging have been performed in tissue culture cells, where packaged pgRNA can be distinguished from cytoplasmic RNAs by its protection from ribonuclease digestion. Efforts to reconstitute this reaction under cell-free conditions *in vitro* have been unsuccessful so far. However, the ability to express an active RT of DHBV using the rabbit reticulocyte lysate (RRL) *in vitro* translation system (10, 11), and the

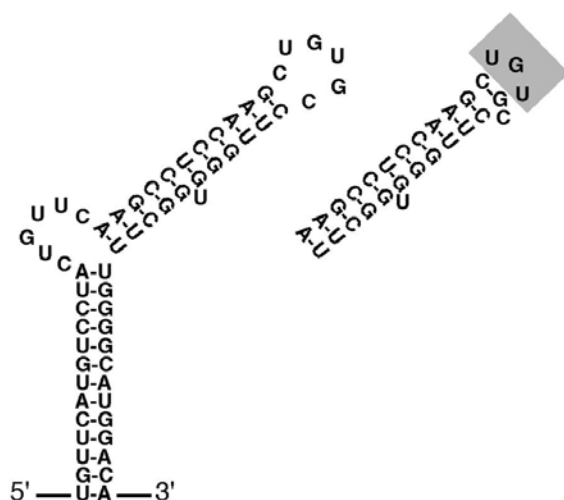
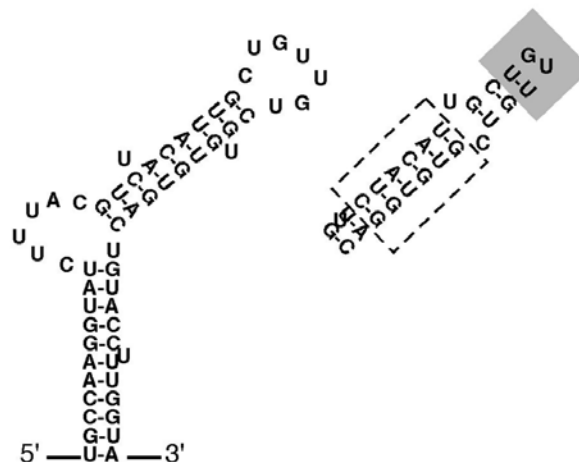
A. HBV**B. DHBV**

Figure 2. Proposed structures for the RNA packaging signal ϵ . The stem-loop structures for the HBV (A) and DHBV (B) ϵ RNA. The left scheme in each case represents the structures as originally proposed by Junker-Niepmann, *et al* (7) based on phylogenetic analysis and secondary structure prediction. The recently solved structures (by NMR) of the upper portion of the HBV and DHBV ϵ are shown to the right for comparison. Shaded boxes denote the apical loops as determined by NMR. The dash-lined box denotes the unstable base pairing in the middle of the upper stem of DHBV ϵ .

subsequent success in reconstituting both DHBV and HBV RT- ϵ interaction using purified components (12-15), have provided feasible experimental systems for detailed biochemical and genetic studies of the RT- ϵ interaction.

3.1. pgRNA determinants

3.1.1. ϵ RNA

The pgRNA packaging signal, ϵ , bears two inverted repeats and forms a stem-loop structure that is phylogenetically conserved among all hepadnaviruses. Secondary structure prediction initially suggested that the HBV ϵ RNA featured a lower and an upper stem of 13 and 11 nucleotides in length, respectively, and an apical loop and an internal bulge, both of which spanned six nucleotides (Figure 2) (7, 16). In addition, the upper stem bears a single bulged U residue. Some evidence for the proposed structure in solution was obtained by RNase mapping, and chemical probing experiments (17, 18), and more recently, by nuclear magnetic resonance (NMR) studies (see below). In addition, extensive mutagenesis studies have, in general, confirmed the importance of the proposed ϵ structure in viral RNA packaging and DNA synthesis (17-21). The nature of the cis-acting regions of ϵ for efficient pgRNA packaging can be summarized as follows. First, the lower stem and the internal bulge appear to play mainly a structural role, the sequence of which can be altered without significant effect on RNA packaging. Second, the specific sequences on the lower right side of the upper stem are required. Third, sequences of the apical loop, in contrast to the internal bulge, contribute critically, in a sequence specific manner, to RNA packaging.

The availability of an active RT protein of DHBV, expressed in a cell free system, provided the first

opportunity to examine directly the determinants of ϵ that dictate its specific interaction with RT (10, 11, 20, 22, 23). It has been demonstrated that the DHBV RT, expressed *in vitro*, forms a stable RNP complex with ϵ . As expected, these *in vitro* RNA binding studies have confirmed that the specific interaction between the RT and ϵ is a prerequisite for RNA packaging and DNA synthesis as determined in cell cultures. Thus, the requirements of ϵ for efficient RT binding, in general, mimic those described above for pgRNA packaging. However, exceptions have been found. Most notably, certain specific sequences of the apical loop of ϵ , while critical for RNA packaging, may not be required for DHBV RT binding (20, 22, 23).

For reasons that are still unknown, the HBV RT protein expressed in the RRL, the same *in vitro* translation system that can express a highly active DHBV RT, has not displayed any activity in either binding to the HBV ϵ RNA or in DNA synthesis (see Section 2 below). However, recent success in reconstituting a functional HBV RT with purified viral and cellular components (see Section 4 below) that is active in specific recognition of the cognate HBV ϵ RNA (13, 14) has allowed for the detailed analysis of both the RNA and protein determinants required for HBV RT- ϵ interactions. The ϵ RNA determinants required for RT binding *in vitro* in HBV are in general similar to those defined earlier for DHBV, including the structural requirements for the internal bulge and both sequence and structure determinants of the lower and upper stem. However, in sharp contrast to DHBV, the entire apical can be deleted without affecting *in vitro* RT binding (Figure 3). As the apical loop is known to be required for pgRNA packaging in cells, these results suggest that the apical loop may interact with yet-to-be identified host factors, instead

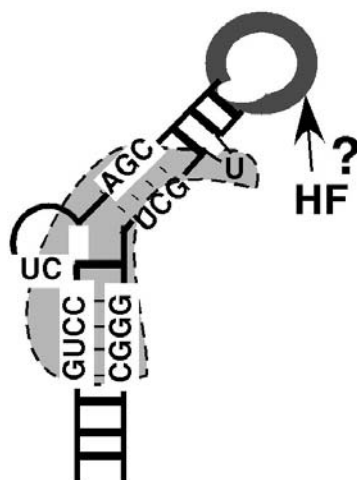


Figure 3. Model of HBV ϵ binding by RT and putative host factor. The HBV ϵ RNA is depicted as the typical stem loop structure. Critical sequences for RT binding (bounded by the dotted line) center around the internal bulge, including the first two nucleotides of the bulge, the upper portion of the lower stem and the lower portion of the upper stem. The apical loop, dispensable for RT binding but critical for pgRNA packaging, may interact with unknown host factor (s) (HF) in directing pgRNA packaging, and possibly protein priming. Adapted from Hu and Boyer (13). See text for detailed discussions of the model.

of the RT protein, to facilitate pgRNA packaging, and possibly also the initiation of viral DNA synthesis (Section 2 below). Another apparent difference in the RNA determinants required for RT binding between HBV and DHBV is the critical role of the first two nucleotides of the internal bulge of HBV, but not DHBV, ϵ .

It was initially thought that the HBV ϵ bulge played mainly a structural role in pgRNA packaging in that certain bulge substitutions still allowed efficient pgRNA packaging (17-19, 24). However, some specific bulge sequences were known to be required for pgRNA packaging. In particular, the first two nucleotides (CU) of the bulge were strongly selected for in a reiterative selection procedure for packaging competent ϵ RNA variants (25). In nature, the entire bulge sequence is highly conserved (18, 25-27). The only naturally occurring variation reported at the first two positions is the less drastic C-U transition at the first position (27), which was, interestingly, also shown to retain some residual *in vitro* RT binding activity (13) and was also selected for, at a lower frequency, in the RNA selection study (25). The conservation of the 3' four nucleotides of the bulge underscores their role as the template for viral DNA synthesis (see Section 2.1 later). A base specific recognition of the 5' two nucleotides of the ϵ bulge by RT provides a nice explanation for the sequence conservation at these two positions, which, by virtue of their specific interaction with RT, plays a critical role in pgRNA packaging.

A high-resolution structure of the ϵ RNA is still not yet available. However, recent efforts in this direction have begun to provide a clearer picture of ϵ structure than before. The NMR technique has recently been used to study the structure of the upper portion, i.e., the upper stem and the apical loop, of the HBV and DHBV ϵ and the "primer loop" (i.e., the internal bulge plus the adjacent base pairs in the upper and lower stems) of the DHBV ϵ . The structure obtained is in general agreement with the previous models based on secondary structural prediction, phylogenetic analyses, and enzymatic and chemical probing. However, some differences have been found. The upper most base pair of the predicted lower stem of the DHBV ϵ may be absent or not stably formed (28). Furthermore, the NMR studies reveal that the apical loop, initially proposed to be 6-nt long, may actually be shorter than predicted in both HBV and DHBV. Thus, in the HBV ϵ , the NMR data indicates the apical loop is capped by a 3-nt UGU tri-loop that is closed by a C-G base pair at its base and followed by a single unpaired and bulged-out C residue (Figure 2) (26, 29). In the DHBV ϵ , the apical loop folds into a well-defined UGUU tetra-loop with a non-canonical U-U base pair stacked onto the closing C-G base pair (Figure 2) (28). In addition, thermodynamic analyses suggest that the middle portion of the upper stem of DHBV ϵ is unstable and may melt under physiological conditions. These results may provide an explanation for the puzzling observation that the DHBV RT can recognize both the DHBV ϵ and the ϵ RNA from another avian hepadnavirus, the heron HBV (HHBV) (11). Secondary structure prediction and biochemical analyses had suggested that unlike the predicted DHBV ϵ structure that contained a well-structured upper stem, the upper stem of the HHBV ϵ would be disrupted (30). It appears that the structural instability or flexibility of the upper stem may instead be a common feature of the avian ϵ RNA recognized by the RT protein (28, 31).

Indeed, there is evidence to suggest that both the ϵ RNA and the RT protein undergo structural changes upon RNP formation. Thus, upon binding to RT, both the DHBV and HHBV ϵ may adopt a new common structure by an induced-fit mechanism common to RNA-protein interactions (22, 23). Similarly, the DHBV RT protein also appears to change its conformation upon binding to its cognate ϵ RNA (32, 33). No information is yet available about any potential conformational changes in the HBV RT- ϵ interaction.

3.1.2. Two regions are required for DHBV pgRNA packaging

In contrast to HBV where the short ϵ RNA signal (together with a 5' cap structure, see below) is sufficient to mediate RNA packaging, a rather long (more than 1 kb) segment of the DHBV pgRNA including the ϵ element was found to be required for DHBV pgRNA packaging (9). Subsequently it was shown that in addition to ϵ , a second region (so-called region II or pac 2, Figure 1), located approximately 1 kb downstream from ϵ , plays a critical role in RNA packaging (8, 34). The intervening sequence between ϵ and pac 2 also appears to contribute to pgRNA

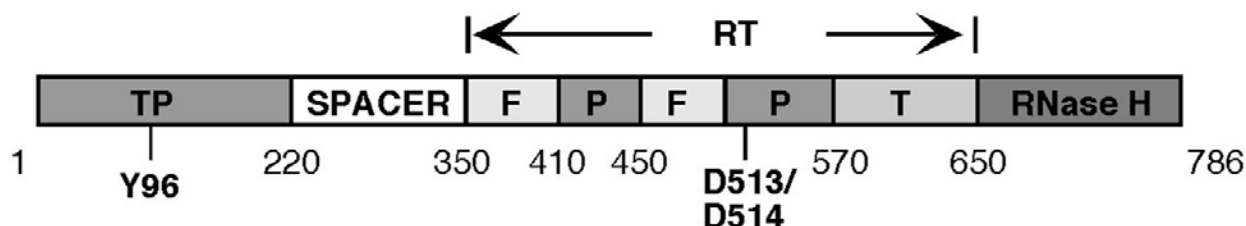


Figure 4. Domain structures of the hepadnavirus RT. The DHBV RT domains are shown schematically, with the approximate boundaries between the domains indicated. The central RT domain is further divided into the fingers (F), palm (P), and thumb (T) sub-domains through alignment with retroviral RT proteins. The primer tyrosine (Y96) in the TP domain and two catalytic aspartate residues (D513 and D514) in the highly conserved (among all RTs) YMDD motif of the palm sub-domain are indicated. Adapted from Wang *et al* (49).

packaging as well. There is currently no explanation as to why only DHBV, but not HBV, requires a second region for RNA packaging, nor is it clear how pac 2 functions in pgRNA packaging. Pac 2 could function by interacting with the viral RT and/or host RNA binding proteins, or it may play a structural role by supporting the formation of an appropriate RNA conformation competent for packaging.

3.1.3. The pgRNA cap

As mentioned above, only the 5' copy of ϵ on pgRNA is functional in mediating RNA packaging whereas the 3' copy is not. Thus, all HBV (and DHBV) RNAs, including the surface mRNAs and X mRNA, in addition to pgRNA, harbor the 3' ϵ but only pgRNA is packaged into nucleocapsids. Efforts to understand this functional difference of the two copies of ϵ led to the realization that the distance between the 5' cap of the RNA and ϵ was critical for ϵ to mediate RNA packaging; A distance of more than 65 nt resulted in a loss of the RNA packaging function (35). Furthermore, in the absence of the cap, ϵ was unable to direct RNA packaging. These results suggest that the 5' proximal ϵ , together with the nearby cap structure, are both required to mediate RNA packaging. How the cap functions in mediating RNA packaging is not yet understood but it could act through binding to the RT protein and/or cellular cap binding proteins, which could, in turn, interact with RT or the viral core protein (Section 3 below). It is not yet known whether a similar cap requirement exist for DHBV RNA packaging.

3.2. The RT protein determinants

Comparison of the predicted amino acid sequence of the hepadnavirus RT protein with those of retroviruses, as well as results from genetic experiments, allowed the assignment of specific functions to the structural domains of the polypeptide (Figure 4) (21, 36-39). The central and C-terminal regions constitute the DNA polymerase (RT) and RNase H domains, which share significant homologies with the corresponding domains of other RTs. By contrast, the N-terminal domain (terminal protein, or TP) does not share any significant homology with other known polypeptides. It is separated by a tether (spacer) from the polymerase domain and bears a tyrosine residue that acts as the primer for RNA-directed DNA synthesis (see Section 2.1 below). Early genetic studies showed that a structurally intact RT protein, including all its structural domains, is

required for RNA packaging (4-6, 40). On the other hand, no enzymatic activity of RT is required. Similar observations were made in the *in vitro* system where the RT- ϵ interaction can be studied independently of RNA packaging (11, 20). Differences between RNA packaging *in vivo* and RT- ϵ binding *in vitro* became apparent with C-terminal deletions of the RT that abolish RNA packaging but seem not to affect ϵ binding (11, 13, 14, 20). The analyses of more than 50 variants of the DHBV polymerase with single amino acid changes point to two regions with conserved arginine residues that are critical for RT- ϵ interaction (38). These map to the TP and the RT domains, further supporting the role of these two domains in ϵ binding and RNA packaging. Similarly, approximately 150 amino acid residues from the TP and 230 from the RT domain of the HBV RT are sufficient for ϵ binding *in vitro* (13, 14). Removal of the entire RNase H and the C-terminal one third of the RT domain of the HBV RT protein, including the catalytic YMDD motif, does not affect ϵ binding *in vitro*. All these results support a model whereby the RT polypeptide acts as a scaffold for the assembly of nucleocapsids, with residues from the TP and RT domains contacting ϵ and perhaps, the C-terminal domain interacting with the core proteins, either directly or indirectly. Thus it is clear that for both RT and ϵ , more determinants are needed for *in vivo* RNA packaging than for *in vitro* RNA-protein interaction. A better understanding of the role of the different domains of RT in pgRNA packaging awaits the development of cell-free systems of pgRNA packaging where the different protein-RNA and protein-protein interactions can be studied in detail.

4. RT-pgRNA INTERACTION IS REQUIRED FOR VIRAL DNA SYNTHESIS

4.1. Initiation of reverse transcription – protein priming

Unique among all known DNA polymerases, the hepadnavirus RT initiates DNA synthesis using a novel protein priming mechanism whereby a specific tyrosine residue within the TP domain of the RT protein itself is used as a primer to initiate DNA synthesis (10, 41-44). Thus, the DNA polymerase activity and the protein primer reside on the same polypeptide, in contrast to other known protein priming reactions (e.g., in adenovirus for DNA synthesis and picornaviruses for RNA synthesis) where the protein primer and polymerase activity reside on separate

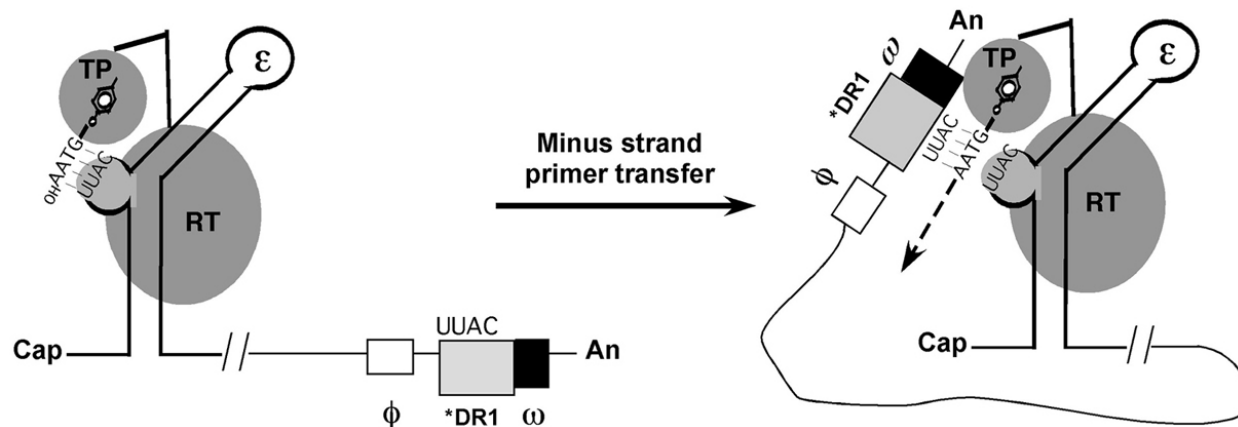


Figure 5. Protein priming and minus strand DNA transfer reactions in hepadnaviruses. Shown schematically are the ϵ stem-loop at the 5' end of pgRNA and the RT protein with its TP and RT domains. (The RNase H domain is omitted for clarity.) Also shown are the RNA elements, ϕ , *DR1, and ω , which are involved in specifying the acceptor site on the 3' end of pgRNA during minus strand DNA transfer following protein priming. Initiation of reverse transcription is triggered by the formation of the RNP complex between ϵ and RT. A specific tyrosine residue located at the TP domain acts as the primer for minus strand DNA synthesis. Using the 3' 4 nucleotides of the ϵ bulge (UUAC in DHBV) as a template, RT synthesizes a short DNA oligomer (GTAA), which becomes covalently attached to RT via the primer tyrosine residue (protein priming). Subsequently, a template switch occurs whereby the nascent minus DNA-RT complex is translocated to the 3' end of pgRNA. The nascent DNA strand then anneals to the homologous sequences at the acceptor site (*DR1) and minus strand DNA elongation continues. Long-range base-pairing interactions among sequences within ϵ , ϕ and ω may help to specify the acceptor site during minus strand DNA transfer. See text for details.

proteins (45). Furthermore, the ϵ RNA serves as an obligate template for the protein priming reaction in hepadnaviruses. Specifically, the 3' four nucleotides of its internal bulge template the synthesis of the first four nucleotides of the viral minus strand DNA, which becomes covalently attached to the RT protein via the primer tyrosine residue as a result of the protein priming mechanism (Figure 5) (19, 46-49). In fact, the RT protein remains attached to the 5' end of the minus strand DNA during the entire process of viral DNA synthesis until it is removed by an unknown mechanism following infection during the process of CCC DNA formation (50, 51). Thus, ϵ serves a critical dual function in viral replication, both as the RNA packaging signal and the origin of reverse transcription.

A comparison of the ε and RT determinants required for RNA-protein interaction vs. those required for protein priming indicates that, as expected, ε -RT interaction is a prerequisite for protein priming (11, 20). In general the determinants required for protein priming are similar to what is described above for ε -RT interaction. However, some mutations of the DHBV ε RNA that do not affect RT binding nevertheless abolish its function in protein priming (20, 22, 23) or pgRNA packaging (Section 1.1.1 above). Thus, “physical” binding of ε to RT may not be sufficient to support either protein priming or RNA packaging. This has led to the suggestion that only “functional” binding between the RT protein and ε RNA is able to facilitate protein priming. One deficiency of the mere physical binding, as opposed to functional binding, might be the inability to trigger the induced-fit type of conformational changes in either the RT, which may be

required for it to develop enzymatic activity (32, 33) or in the ϵ RNA, which may be required for it to serve as a functional template for protein priming (22, 23, 31). HBV protein priming, in an ϵ -dependent manner, has not been reproduced *in vitro* and it is not yet feasible to directly visualize protein priming *in vivo* (in the absence of additional DNA strand elongation). Consequently, it is difficult to correlate the requirements of RT- ϵ interaction with those for protein priming in HBV. However, a comparison of the ϵ determinants required for RT binding *in vitro* vs. those required for viral DNA synthesis *in vivo* suggests that recognition of some ϵ sequences, including the sequence at the left side of the lower portion of the upper stem, by the HBV RT may be required specifically for viral DNA synthesis but not for pgRNA packaging (13).

4.2. Minus strand DNA elongation

To continue minus strand DNA synthesis following protein priming, the 4-nt long nascent minus strand DNA, covalently attached to the RT protein, dissociates from ϵ and is translocated to an acceptor site within the 3' copy of the direct repeat 1 on pgRNA (*DR1, Figure 5), where the nascent minus strand DNA anneals to complementary sequences (47, 52, 53). DNA strand elongation then ensues from this new position. It is unclear what triggers the arrest of DNA synthesis following the synthesis of the 4-nt long DNA oligomer. *In vitro*, a portion of the nascent DNA can be elongated *in situ* from ϵ (10, 53). It is conceivable that the first (5') two nucleotides of the ϵ bulge may be inaccessible to the RT active site due to structural features of the lower stem and/or of the bulge itself or to tight binding by the polymerase (13, 25).

Interestingly, a similar problem is encountered by telomerases, which also copy only a small portion of its RNA template into DNA (54).

How the acceptor site at the 3' end of pgRNA is selected is also not clearly understood. The short (4-nt) homology between the donor (the ϵ bulge) and acceptor (*DR1) sites on the pgRNA template contributes to this selection but is apparently not sufficient to specify the acceptor sites (52, 53, 55). There are numerous other potential acceptor sites with the same 4-nt homology on pgRNA, which are not used during minus strand template switch. Recent studies indicate specific sequence elements on pgRNA in the vicinity of the acceptor site, both upstream (the ϕ element) and downstream (the ω element), cooperate in specifying the acceptor site selection (Figure 1 and 5) (56-58). These elements have the potential to form complex, long-range base pairing interactions with the 5' ϵ structure and thus, can circularize pgRNA and bring the donor and acceptor sites, which are separated by 3 kb in linear sequence, into spatial proximity to facilitate template switching (56, 59, 60). Whether these RNA elements function by interacting with any proteins, the viral RT or core protein or cellular proteins, is unknown at present.

An important consequence of the protein priming mechanism for initiating viral DNA synthesis in hepadnaviruses is that the ϵ -RT interaction has to be transient in nature and that conformational changes in either RT or ϵ or both likely have to occur to promote the transition from protein priming to DNA strand elongation. In particular, the bulge of ϵ , which occupies the active site of RT, must be displaced by sequences from the 3' end of pgRNA, which provide the template for subsequent elongation of minus strands. Evidence in support of a conformational change in RT following protein priming has been provided by the observation that the pyrophosphate analog, phosphonoformic acid, and most nucleoside analogs can block minus strand DNA elongation but have no effect on protein priming (10, 61). Also, a structural rearrangement of the ϵ sequences, following protein priming, is suggested by the long-range interaction between part of the sequences within ϵ and ϕ , as described above. Thus, this long-range RNA-RNA interaction would disrupt the ϵ fold, facilitate the dissociation of RT from the disrupted ϵ structure, and stimulate minus strand DNA transfer. However, how the sequences within ϵ participate in the two exclusive RNA structures at the two different stages of reverse transcription remains unclear. Therefore, relatively little is known about the exact nature of the structural dynamics that is likely to occur during the minus strand transfer reaction. Investigations of these events are hampered, in part, by the low efficiency and specificity of the transfer reaction in the *in vitro* protein priming reaction (10, 53). It is possible that this process depends on the formation of replication-competent nucleocapsids, which has yet to be modeled in any cell-free systems, and a direct involvement of the viral core protein, which forms the nucleocapsid shell (see Section 3 below).

5. THE CORE PROTEIN PLAYS AN ACTIVE ROLE IN PGRNA PACKAGING AND DNA SYNTHESIS

Genetic studies in both HBV and DHBV have demonstrated that the viral core protein plays an active role in viral RNA packaging and reverse transcription. The core protein can be divided into two different functional domains, with the N-terminal two thirds responsible for assembly into the capsid shell and the C-terminal one third participating actively in RNA packaging and DNA synthesis (62-68). The C-terminal domain is highly basic (rich in arginines and called protamine-like) and has non-specific DNA and RNA binding activities (69-71). Furthermore, the C-terminal domain is phosphorylated at multiple S/T sites, whose dynamic phosphorylation and dephosphorylation play a critical role in both RNA packaging and DNA synthesis (72-78). Exactly how the core protein C-terminal domain, and its phosphorylation state, function in RNA packaging and DNA synthesis is not clearly understood. Its positive charges likely help to neutralize the negative charges of the viral RNA and DNA, and its RNA and DNA binding activity may help to condense and organize the viral pregenome and genome and thus facilitate RNA packaging and DNA synthesis, similar to the role of cellular histone proteins in the nucleus. However, whether and how the core protein facilitates viral RNA packaging and DNA synthesis beyond simple charge neutralization requires further investigation.

6. SPECIFIC HOST FACTORS ARE INVOLVED IN NUCLEOCAPSID ASSEMBLY AND REVERSE TRANSCRIPTION

Although only two viral proteins (the RT and core) and one RNA (pgRNA) are required for hepadnavirus reverse transcription, it has become increasingly clear that multiple host factors play important roles at various stages of viral replication. In particular, recent studies have identified a number of host chaperone proteins as playing a critical role in facilitating the interaction between the viral RT protein and pgRNA.

6.1. Host chaperones are required for RT- ϵ interaction

It has long been recognized that the hepadnavirus RT is a difficult protein to express, purify and characterize (37). To this day, efforts to obtain large quantities of a highly purified RT protein for structural and detailed biochemical analyses remain unsuccessful despite numerous attempts. The first breakthrough in the biochemical analysis of RT function came when the DHBV RT was expressed in the RRL *in vitro* translation system as an active protein functional in specific ϵ binding and protein priming (10). The RRL system thus allowed for rather detailed analyses of the requirements for RT- ϵ interaction and protein-primed initiation of reverse transcription in a cell-free system, as detailed above.

Failure to express a similarly active RT using an alternate *in vitro* translation system, the wheat germ extract, led to the first realization that specific cellular factors, functional in the RRL but deficient in the wheat germ extract, may be required for RT functions (Figure 6) (79).

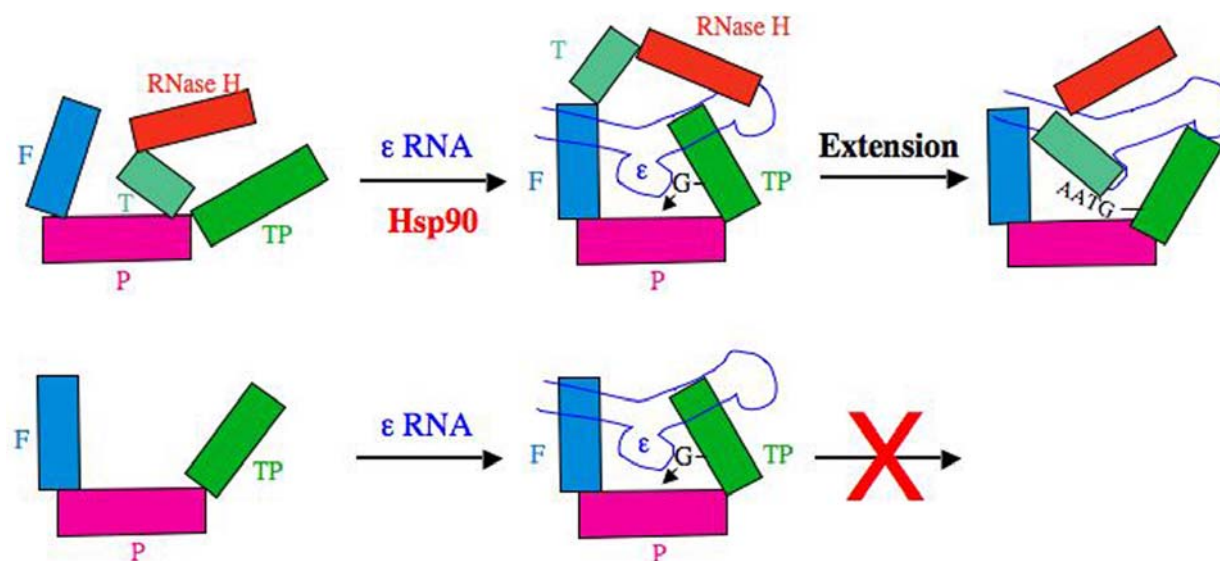


Figure 6. Working model for Hsp90-dependent and -independent folding of the DHBV RT and MiniRT2. The domains and sub-domains of RT are depicted as blocks. Notations are as in Figure 4. The ϵ RNA is depicted as a stem-loop structure, with its internal bulge (the template for protein priming) facing the RT palm sub-domain. In the case of the full-length RT, the thumb sub-domain (and the RNase H domain) may prematurely interact with the palm sub-domain and preclude the TP domain from accessing the RT active site to establish a conformation competent for ϵ binding and initiation of protein priming. The Hsp90 chaperone complex is proposed to counteract this inhibitory effect of the thumb sub-domain and RNase H domain by preventing these inappropriate interactions and may, additionally, facilitate the productive interactions between the TP and the RT domains. In the case of MiniRT2, the removal of the thumb sub-domain and RNase H domain allows the mini RT to fold independently of Hsp90. On the other hand, following the initiation of protein priming (the covalent linkage of the dGMP residue to the TP domain), the thumb sub-domain has to access the RT active site (and the TP has to exit from it) in order to facilitate the subsequent DNA extension leading to the synthesis of the nascent DNA oligomer, GTAA. Lacking the thumb sub-domain, MiniRT2 is thus unable to carry out any DNA elongation. Adapted from Wang *et al* (49). See text for details.

Efforts to identify these putative host factors led to a group of molecular chaperone proteins, which include the 90 kd heat shock protein (Hsp90) and several co-chaperones, Hsp70, Hsp40, Hop/p60, and p23 (15, 79, 80). Initial studies relying on the use of specific monoclonal antibodies and pharmacological inhibitors demonstrated that the Hsp90 complex was functionally required for DHBV RT- ϵ interaction and thus, for protein priming *in vitro* and RNA packaging and DNA replication in cells (79, 80). This chaperone complex associates with the DHBV RT translated in the RRL and, in a dynamic process that requires ATP hydrolysis, helps to establish and maintain the RT protein in a conformation competent for ϵ binding. The definition of the minimal DHBV RT sequences required for ϵ binding and protein priming, using the RRL system, also led to the successful expression and purification of truncated mini DHBV RT proteins using the bacterial expression system, which allows the purification of RT proteins at much higher levels than that achievable in the RRL (12, 81). Biochemical reconstitution experiments using these purified mini RT proteins and host factors, both in the form of cell lysate and purified proteins, eventually led to the identification of the essential components of the Hsp90 complex required for DHBV RT- ϵ interaction *in vitro* (12, 15, 81). These efforts culminated in the establishment of a defined reconstitution system using the five chaperone/co-chaperone proteins mentioned above,

which is nearly as efficient as the RRL in stimulating RT- ϵ interaction and protein priming (15). Less efficient reconstitution of RT- ϵ interaction can also be achieved using sub-components of the chaperone system, in particular, Hsp70 plus Hsp40 (15, 82).

Further studies using both the RRL and the defined reconstitution system led to the isolation of a DHBV mini RT protein, MiniRT2, which no longer requires the cellular chaperones in order to interact with ϵ and carry out protein priming (49). The severely truncated MiniRT2, with deletions of the N-terminal part of the TP domain, the entire RNase H domain, the C-terminal portion of the central RT domain (the putative thumb sub-domain), as well as the spacer region (Figure 4 & 6), retains only half of the original RT sequences. MiniRT2, as purified from bacteria, is active in specific ϵ binding and protein priming *in vitro*, without the need for any host proteins. The isolation of this chaperone-independent mini RT protein suggests that the mechanism of action of the Hsp90 complex in facilitating the full-length RT folding and functions is to counteract the action of the RT sequences (i.e., those deleted in MiniRT2, particularly the C-terminal RNase H domain) that function in an auto-inhibitory fashion against RT- ϵ interaction and protein priming but are nevertheless required for pgRNA packaging and later steps in viral DNA synthesis (Figure 6). The help of the

cellular chaperones is thus needed for the viral RT protein to carry out its multiple essential functions at the different stages of viral assembly and replication.

The role of the Hsp90 complex in facilitating RT- ϵ interaction *in vitro* and pgRNA packaging *in vivo* has recently been demonstrated for HBV as well, including the establishment of a defined reconstitution system for HBV RT- ϵ interaction *in vitro* (13, 14). However, for reasons still unknown, the HBV RT- ϵ complex formed *in vitro* is inactive in protein priming. This and other results suggest that additional host factors may be required for protein priming specifically by HBV (but not DHBV), perhaps via binding to the HBV ϵ RNA (see Section 4.2 below).

6.2. Other host factors

The finding that certain substitutions of the DHBV ϵ apical loop sequence decreased protein priming and RNA packaging but not RT binding led to the initial suggestion that a putative host factor may bind to the apical loop and play an active role in stimulating protein priming and RNA packaging (20). Recent success in the reconstitution of DHBV protein priming with purified components indicates that such a cellular ϵ binding factor, if it exists, is not essential for DHBV protein priming (15, 49, 82). On the other hand, the failure of HBV RT to carry out protein priming either in the RRL or in the defined reconstitution system, despite its ability to bind ϵ , suggests that additional host factors may indeed be required for HBV protein priming, beyond the Hsp90 complex that is required for RT- ϵ interaction (13). As described above, a surprising finding from the HBV RT- ϵ interaction studies is that the apical loop of the HBV ϵ RNA is entirely dispensable for RT binding *in vitro*, even though the same sequence is clearly required for pgRNA packaging and presumably for protein priming. Thus, as originally suggested for DHBV, a host factor may indeed bind to the HBV ϵ apical loop and facilitate HBV protein priming (Figure 3). On the other hand, alternative explanations are possible. The specific sequences of the apical loop may affect some aspect of the ϵ RNA structure, which, although important for RNA packaging and protein priming, functions independently of any protein interactions. For example, the loop mutations may prevent (or fail to induce) the conformational changes in ϵ and/or RT that are thought to be critical for protein priming and RNA packaging, i.e., they may act by blocking the transition from physical binding to functional binding between RT and ϵ .

The cellular cytidine deaminase, Apobec3G, and its relatives have recently been shown to block reverse transcription of hepadnaviruses as well as conventional retroviruses (83-85). Initially thought to act exclusively through lethal mutagenesis via DNA deamination, Apobec3G has been shown to inhibit viral replication through both deamination dependent and independent mechanisms. In fact, the deamination-independent mechanism turns out to be the predominant way by which Apobec3G inhibits HBV replication (85, 86). On the other hand, a related cytidine deaminase, Apobec3C, seems to be able to inhibit HBV replication mainly by editing of the

viral DNA (87). Apobec3G is known to bind non-specifically to viral and cellular RNAs, which may facilitate its incorporation into viral particles. In HBV, the potential interaction between Apobec3G and the viral pgRNA may underlie its potent inhibitory effect on HBV reverse transcription, which is blocked at a very early stage by this cellular antiviral protein (86).

7. PERSPECTIVES

The presence of multiple cis-acting sequences on the hepadnavirus pgRNA reflects its multiple roles in viral assembly and replication. Except for the ϵ -RT interaction, little information is available about the nature of potential RNA-protein interactions that have to take place in order for pgRNA to carry out its multiple functions during RNA packaging and reverse transcription. Nevertheless, the identification and characterization of the various cis-acting sequences on pgRNA will now facilitate the isolation of putative trans-acting proteins, viral or cellular, that specifically bind to these RNA sequences. Future efforts to further identify and characterize these protein factors binding to pgRNA will not only provide important insights into the molecular mechanisms of hepadnavirus reverse transcription but may also bring novel insights into RNA-protein interactions in general.

8. ACKNOWLEDGMENTS

Work in the authors' laboratory has been supported by a Public Health Service Grant, R01 AI43453, from the National Institute of Health (to J. H.) and an American Cancer Society Research Scholar Grant, RSG MBC-108387 (to J. H.).

9. REFERENCES

1. Summers, J. & W. S. Mason: Replication of the genome of a hepatitis B-like virus by reverse transcription of an RNA intermediate. *Cell*, 29, 403-15 (1982)
2. Seeger, C. & W. S. Mason: Hepatitis B virus biology. *Microbiol Mol Biol Rev*, 64, 51-68 (2000)
3. Seeger, C. & J. Hu: Why are hepadnaviruses DNA and not RNA viruses? *Trends Microbiol*, 5, 447-50 (1997)
4. Bartenschlager, R., M. Junker-Niepmann & H. Schaller: The P gene product of hepatitis B virus is required as a structural component for genomic RNA encapsidation. *J Virol*, 64, 5324-5332 (1990)
5. Bartenschlager, R. & H. Schaller: Hepadnaviral assembly is initiated by polymerase binding to the encapsidation signal in the viral RNA genome. *EMBO J*, 11, 3413-20 (1992)
6. Hirsch, R. C., J. E. Lavine, L. J. Chang, H. E. Varmus & D. Ganem: Polymerase gene products of hepatitis B viruses are required for genomic RNA packaging as well as for reverse transcription. *Nature*, 344, 552-5 (1990)

7. Junker-Niepmann, M., R. Bartenschlager & H. Schaller: A short cis-acting sequence is required for hepatitis B virus pregenome encapsidation and sufficient for packaging of foreign RNA. *EMBO J*, 9, 3389-96 (1990)
8. Calvert, J. & J. Summers: Two regions of an avian hepadnavirus RNA pregenome are required in cis for encapsidation. *J Virol*, 68, 2084-90 (1994)
9. Hirsch, R. C., D. D. Loeb, J. R. Pollack & D. Ganem: cis-acting sequences required for encapsidation of duck hepatitis B virus pregenomic RNA. *J Virol*, 65, 3309-16 (1991)
10. Wang, G. H. & C. Seeger: The reverse transcriptase of hepatitis B virus acts as a protein primer for viral DNA synthesis. *Cell*, 71, 663-70 (1992)
11. Wang, G. H., F. Zoulim, E. H. Leber, J. Kitson & C. Seeger: Role of RNA in enzymatic activity of the reverse transcriptase of hepatitis B viruses. *J Virol*, 68, 8437-42 (1994)
12. Hu, J. & D. Anselmo: In vitro reconstitution of a functional duck hepatitis B virus reverse transcriptase: posttranslational activation by Hsp90. *J Virol*, 74, 11447-55 (2000)
13. Hu, J. & M. Boyer: Hepatitis B virus reverse transcriptase and epsilon RNA sequences required for specific interaction in vitro. *J Virol*, 80, 2141-50 (2006)
14. Hu, J., D. Flores, D. Toft, X. Wang & D. Nguyen: Requirement of heat shock protein 90 for human hepatitis B virus reverse transcriptase function. *J Virol*, 78, 13122-31 (2004)
15. Hu, J., D. Toft, D. Anselmo & X. Wang: In vitro reconstitution of functional hepadnavirus reverse transcriptase with cellular chaperone proteins. *J Virol*, 76, 269-79 (2002)
16. Hu, J. & C. Seeger: RNA signals that control DNA replication in hepadnaviruses. *Semin Virol*, 8, 205-211 (1997)
17. Knaus, T. & M. Nassal: The encapsidation signal on the hepatitis B virus RNA pregenome forms a stem-loop structure that is critical for its function. *Nucl Acids Res*, 21, 3967-3975 (1993)
18. Pollack, J. R. & D. Ganem: An RNA stem-loop structure directs hepatitis B virus genomic RNA encapsidation. *J Virol*, 67, 3254-63 (1993)
19. Fallows, D. A. & S. P. Goff: Mutations in the epsilon sequences of human hepatitis B virus affect both RNA encapsidation and reverse transcription. *J Virol*, 69, 3067-73 (1995)
20. Pollack, J. R. & D. Ganem: Site-specific RNA binding by a hepatitis B virus reverse transcriptase initiates two distinct reactions: RNA packaging and DNA synthesis. *J Virol*, 68, 5579-87 (1994)
21. Radziwill, G., W. Tucker & H. Schaller: Mutational analysis of the hepatitis B virus P gene product: domain structure and RNase H activity. *J Virol*, 64, 613-20 (1990)
22. Beck, J. & M. Nassal: Sequence- and structure-specific determinants in the interaction between the RNA encapsidation signal and reverse transcriptase of avian hepatitis B viruses. *J Virol*, 71, 4971-4980 (1997)
23. Beck, J. & M. Nassal: Formation of a functional hepatitis B virus replication initiation complex involves a major structural alteration in the RNA template. *Mol Cell Biol*, 18, 6265-72. (1998)
24. Tong, S.-P., J.-S. Li, L. Vitvitski, A. Kay & C. Trepo: Evidence for a base-paired region of hepatitis B virus pregenome encapsidation signal which influences the patterns of precore mutations abolishing HBe protein expression. *J. Virol.*, 67, 5651-5655 (1993)
25. Rieger, A. & M. Nassal: Distinct requirements for primary sequence in the 5'- and 3'-part of a bulge in the hepatitis B virus RNA encapsidation signal revealed by a combined in vivo selection/in vitro amplification system. *Nucleic Acids Res*, 23, 3909-15 (1995)
26. Flodell, S., J. Schleucher, J. Croomsigt, H. Ippel, K. Kidd-Ljunggren & S. Wijmenga: The apical stem-loop of the hepatitis B virus encapsidation signal folds into a stable tri-loop with two underlying pyrimidine bulges. *Nucleic Acids Res*, 30, 4803-11 (2002)
27. Laskus, T.: The stem-loop structure of the cis-encapsidation signal is highly conserved in naturally occurring hepatitis B virus variants. *Virology*, 200, 809-812 (1994)
28. Girard, F. C., O. M. Ottink, K. A. Ampt, M. Tessari & S. S. Wijmenga: Thermodynamics and NMR studies on Duck, Heron and Human HBV encapsidation signals. *Nucleic Acids Res*, 35, 2800-11 (2007)
29. Flodell, S., M. Petersen, F. Girard, J. Zdunek, K. Kidd-Ljunggren, J. Schleucher & S. Wijmenga: Solution structure of the apical stem-loop of the human hepatitis B virus encapsidation signal. *Nucleic Acids Res*, 34, 4449-57 (2006)
30. Beck, J., H. Bartos & M. Nassal: Experimental confirmation of a hepatitis B virus (HBV) e-like bulge-and-loop structure in avian HBV RNA encapsidation signals. *Virology*, 227, 500-504 (1997)
31. Hu, K., J. Beck & M. Nassal: SELEX-derived aptamers of the duck hepatitis B virus RNA encapsidation signal distinguish critical and non-critical residues for productive initiation of reverse transcription. *Nucleic Acids Res*, 32, 4377-89 (2004)
32. Tavis, J. E. & D. Ganem: Evidence for activation of the hepatitis B virus polymerase by binding of its RNA template. *J Virol*, 70, 5741-50 (1996)

33. Tavis, J. E., B. Massey & Y. Gong: The duck hepatitis B virus polymerase is activated by its RNA packaging signal, epsilon. *J Virol*, 72, 5789-96 (1998)
34. Ostrow, K. M. & D. D. Loeb: Characterization of the cis-acting contributions to avian hepadnavirus RNA encapsidation. *J Virol*, 76, 9087-95 (2002)
35. Jeong, J. K., G. S. Yoon & W. S. Ryu: Evidence that the 5'-end cap structure is essential for encapsidation of hepatitis B virus pregenomic RNA. *J Virol*, 74, 5502-8 (2000)
36. Chang, L. J., R. C. Hirsch, D. Ganem & H. E. Varmus: Effects of insertional and point mutations on the functions of the duck hepatitis B virus polymerase. *J Virol*, 64, 5553-8 (1990)
37. Hu, J. & C. Seeger: Expression and characterization of hepadnavirus reverse transcriptases. *Methods Enzymol*, 275, 195-208 (1996)
38. Seeger, C., E. H. Leber, L. K. Wiens & J. Hu: Mutagenesis of a hepatitis B virus reverse transcriptase yields temperature-sensitive virus. *Virology*, 222, 430-9 (1996)
39. Toh, H., H. Hayashida & T. Miyata: Sequence homology between retroviral reverse transcriptase and putative polymerases of hepatitis B virus and cauliflower mosaic virus. *Nature*, 305, 827-829 (1983)
40. Chen, Y., W. S. Robinson & P. L. Marion: Selected mutations of the duck hepatitis B virus P gene RNase H domain affect both RNA packaging and priming of minus-strand DNA synthesis. *J Virol*, 68, 5232-8 (1994)
41. Lanford, R. E., L. Notvall & B. Beames: Nucleotide priming and reverse transcriptase activity of hepatitis B virus polymerase expressed in insect cells. *J Virol*, 69, 4431-9 (1995)
42. Lanford, R. E., L. Notvall, H. Lee & B. Beames: Transcomplementation of nucleotide priming and reverse transcription between independently expressed TP and RT domains of the hepatitis B virus reverse transcriptase. *J Virol*, 71, 2996-3004 (1997)
43. Weber, M., V. Bronsema, H. Bartos, A. Bosserhoff, R. Bartenschlager & H. Schaller: Hepadnavirus P protein utilizes a tyrosine residue in the TP domain to prime reverse transcription. *J Virol*, 68, 2994-9 (1994)
44. Zoulim, F. & C. Seeger: Reverse transcription in hepatitis B viruses is primed by a tyrosine residue of the polymerase. *J Virol*, 68, 6-13 (1994)
45. Salas, M.: Protein-priming of DNA replication. In: *Ann.Rev.Biochem. Ann.Rev.Inc.*, (1991)
46. Nassal, M. & A. Rieger: A bulged region of the hepatitis B virus RNA encapsidation signal contains the replication origin for discontinuous first-strand DNA synthesis. *J. Virol.*, 70, 2764-2773 (1996)
47. Rieger, A. & M. Nassal: Specific hepatitis B virus minus-strand DNA synthesis requires only the 5' encapsidation signal and the 3'-proximal direct repeat DR1. *J Virol*, 70, 585-9 (1996)
48. Tavis, J. E., S. Perri & D. Ganem: Hepadnavirus reverse transcription initiates within the stem-loop of the RNA packaging signal and employs a novel strand transfer. *J Virol*, 68, 3536-43 (1994)
49. Wang, X., X. Qian, H.-C. Guo & J. Hu: Heat shock protein 90-independent activation of truncated hepadnavirus reverse transcriptase. *J Virol*, 77, 4471-80 (2003)
50. Gao, W. & J. Hu: Formation of Hepatitis B Virus Covalently Closed Circular DNA: Removal of Genome-Linked Protein. *J Virol*, 81, 6164-74 (2007)
51. Tuttleman, J. S., C. Pourcel & J. Summers: Formation of the pool of covalently closed circular viral DNA in hepadnavirus-infected cells. *Cell*, 47, 451-460 (1986)
52. Tavis, J. E. & D. Ganem: RNA sequences controlling the initiation and transfer of duck hepatitis B virus minus-strand DNA. *J Virol*, 69, 4283-91 (1995)
53. Wang, G. H. & C. Seeger: Novel mechanism for reverse transcription in hepatitis B viruses. *J Virol*, 67, 6507-12 (1993)
54. Blackburn, E.: Telomerases. In: *Reverse transcriptase*. Eds: A. Skalka & S. Goff. Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1993)
55. Loeb, D. D. & R. Tian: Transfer of the minus strand of DNA during hepadnavirus replication is not invariable but prefers a specific location. *J Virol*, 69, 6886-91 (1995)
56. Oropeza, C. E. & A. McLachlan: Complementarity between epsilon and phi sequences in pregenomic RNA influences hepatitis B virus replication efficiency. *Virology*, 359, 371-81 (2007)
57. Shin, M. K., J. Lee & W. S. Ryu: A novel cis-acting element facilitates minus-strand DNA synthesis during reverse transcription of the hepatitis B virus genome. *J Virol*, 78, 6252-62 (2004)
58. Tang, H. & A. McLachlan: A pregenomic RNA sequence adjacent to DR1 and complementary to epsilon influences hepatitis B virus replication efficiency. *Virology*, 303, 199-210 (2002)
59. Abraham, T. M. & D. D. Loeb: 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, 4380-7 (2006)

60. Abraham, T. M. & D. D. Loeb: Topology of HBV pregenomic RNA promotes its replication. *J Virol*, 81, 11577-84 (2007)
61. Staschke, K. A. & J. M. Colacino: Priming of duck hepatitis B virus reverse transcription in vitro: Premature termination of primer DNA induced by the 5'-triphosphate of fialuridine. *J Virol*, 68, 8265-8269 (1994)
62. Beames, B. & R. E. Lanford: Carboxy-terminal truncations of the HBV core protein affect capsid formation and the apparent size of encapsidated HBV RNA. *Virology*, 194, 597-607 (1993)
63. Gallina, A., F. Bonelli, L. Zentilin, G. Rindi, M. Muttini & G. Milanesi: A recombinant hepatitis B core antigen polypeptide with the protamine-like domain deleted self assembles into capsid particles but fails to bind nucleic acids. *J Virol*, 63, 4645-4652 (1989)
64. Kock, J., M. Nassal, K. Deres, H. E. Blum & F. von Weizsacker: Hepatitis B virus nucleocapsids formed by carboxy-terminally mutated core proteins contain spliced viral genomes but lack full-size DNA. *J Virol*, 78, 13812-8 (2004)
65. Le Pogam, S., P. K. Chua, M. Newman & C. Shih: Exposure of RNA templates and encapsidation of spliced viral RNA are influenced by the arginine-rich domain of human hepatitis B virus core antigen (HBcAg 165-173). *J Virol*, 79, 1871-87 (2005)
66. Nassal, M.: The arginine-rich domain of the hepatitis B virus core protein is required for pregenome encapsidation and productive viral positive-strand DNA synthesis but not for virus assembly. *J Virol*, 66, 4107-4116 (1992)
67. Wynne, S. A., R. A. Crowther & A. G. Leslie: The crystal structure of the human hepatitis B virus capsid. *Mol Cell*, 3, 771-80 (1999)
68. Yu, M. & J. Summers: A domain of the hepadnavirus capsid protein is specifically required for DNA maturation and virus assembly. *J Virol*, 65, 2511-7 (1991)
69. Hatton, T., S. Zhou & D. Strandberg: RNA- and DNA-binding activities in hepatitis B virus capsid protein: a model for their role in viral replication. *J Virol*, 66, 5232-5241 (1992)
70. Machida, A., O. Tsuda, A. Yoshikawa, Y. Hoshi, T. Tanaka, S. Kishimoto, Y. Akahane, Y. Miyakawa & M. Mayumi: Phosphorylation in the carboxyl-terminal domain of the capsid protein of hepatitis B virus: evaluation with a monoclonal antibody. *J Virol*, 65, 6024-6030 (1991)
71. Yang, W., J. Guo, Z. Ying, S. Hua, W. Dong & H. Chen: Capsid assembly and involved function analysis of twelve core protein mutants of duck hepatitis B virus. *J Virol*, 68, 338-345 (1994)
72. Basagoudanavar, S. H., D. H. Perlman & J. Hu: Regulation of hepadnavirus reverse transcription by dynamic nucleocapsid phosphorylation. *J Virol*, 81, 1641-9 (2007)
73. Gazina, E. V., J. E. Fielding, B. Lin & D. A. Anderson: Core protein phosphorylation modulates pregenomic RNA encapsidation to different extents in human and duck hepatitis B viruses. *J Virol*, 74, 4721-8 (2000)
74. Kann, M. & W. H. Gerlich: Effect of core protein phosphorylation by protein kinase C on encapsidation of RNA within core particles of hepatitis B virus. *J Virol*, 68, 7993-8000 (1994)
75. Melegari, M., S. K. Wolf & R. J. Schneider: Hepatitis B virus DNA replication is coordinated by core protein serine phosphorylation and HBx expression. *J Virol*, 79, 9810-20 (2005)
76. Perlman, D. H., E. A. Berg, B. O'Connor P, C. E. Costello & J. Hu: Reverse transcription-associated dephosphorylation of hepadnavirus nucleocapsids. *Proc Natl Acad Sci U S A*, 102, 9020-5 (2005)
77. Yeh, C. T. & J. H. Ou: Phosphorylation of hepatitis B virus precore and core proteins. *J Virol*, 65, 2327-31 (1991)
78. Yu, M. & J. Summers: Multiple functions of capsid protein phosphorylation in duck hepatitis B virus replication. *J Virol*, 68, 4341-8 (1994)
79. Hu, J. & C. Seeger: Hsp90 is required for the activity of a hepatitis B virus reverse transcriptase. *Proceedings of the National Academy of Sciences of the United States of America*, 93, 1060-4 (1996)
80. Hu, J., D. O. Toft & C. Seeger: Hepadnavirus assembly and reverse transcription require a multi-component chaperone complex which is incorporated into nucleocapsids. *Embo J*, 16, 59-68 (1997)
81. Beck, J. & M. Nassal: Reconstitution of a functional duck hepatitis B virus replication initiation complex from separate reverse transcriptase domains expressed in *Escherichia coli*. *J Virol*, 75, 7410-9. (2001)
82. Beck, J. & M. Nassal: Efficient Hsp90-independent in vitro activation by Hsc70 and Hsp40 of duck hepatitis B virus reverse transcriptase, an assumed Hsp90 client protein. *J Biol Chem*, 278, 36128-38 (2003)
83. Cullen, B. R.: Role and mechanism of action of the APOBEC3 family of antiretroviral resistance factors. *J Virol*, 80, 1067-76 (2006)
84. Sheehy, A. M., N. C. Gaddis, J. D. Choi & M. H. Malim: Isolation of a human gene that inhibits HIV-1

RNA-protein interactions in HBV replication

infection and is suppressed by the viral Vif protein. *Nature*, 418, 646-50 (2002)

85. Turelli, P., B. Mangeat, S. Jost, S. Vianin & D. Trono: Inhibition of hepatitis B virus replication by APOBEC3G. *Science*, 303, 1829 (2004)

86. Nguyen, D. H., S. Gummuluru & J. Hu: Deamination-independent inhibition of hepatitis B virus reverse transcription by APOBEC3G. *J Virol*, 81, 4465-72 (2007)

87. Baumert, T. F., C. Rosler, M. H. Malim & F. von Weizsacker: Hepatitis B virus DNA is subject to extensive editing by the human deaminase APOBEC3C. *Hepatology*, 46, 682-9 (2007)

Key Words: Hepadnavirus, Hepatitis B virus, Hsp90, Protein Priming, Reverse Transcription, RNA packaging, Review

Send correspondence to: Jianming Hu, Department of Microbiology and Immunology, The Penn State University College of Medicine, Hershey, PA 17033, Tel: 717-531-6523, Fax: 717-531-6522, E-mail: juh13@psu.edu

<http://www.bioscience.org/current/vol14.htm>