

CHAPTER 2

Hepatitis Delta Virus:

HDV-HBV Interactions

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Abstract

The hepatitis delta virus (HDV) is a subviral agent that utilizes the envelope proteins of the hepatitis B virus (HBV) for cell to cell propagation. In infected human hepatocytes, the HDV RNA genome can replicate and associate with multiple copies of the delta protein to assemble a ribonucleoprotein (RNP). However the RNP cannot exit the cell because of the lack of an export system. This is provided by the HBV envelope proteins, which are capable of budding at an internal cellular membrane to assemble mature HDV virions when RNPs are present. This review covers advances in the molecular aspects of the HDV-HBV interactions, with an emphasis on the HBV properties that are instrumental in HDV maturation, in particular the central role of the small HBV envelope protein.

Introduction

Since the initial description of the hepatitis delta antigen (HDAg) in 1977 by M. Rizzetto, the viral agent that was later referred to as the “Hepatitis Delta Virus” (HDV), has been clearly related to the Hepatitis B virus (HBV). HDAg was first observed as a new nuclear antigen present only in liver cells of HBV chronic carriers. It was shown to be associated with a viral agent that was transmissible to chimpanzee in the presence of HBV, and at that time, it was considered a defective virus because of its absolute requirement for HBV coinfection. Soon thereafter the HBV helper functions appeared to be limited to providing the protein content of the delta particle envelope, whereas the inner core was found to contain a small RNA molecule bound to HDAg-proteins. The cloning of the HDV-associated RNA was achieved in 1986, and the sequencing analysis revealed a genome structure that was unique among animal viruses: it was a circular, single-stranded RNA of negative polarity, with an open reading frame coding for the HDAg protein (the only protein that HDV RNA is known to encode), but it lacked the coding capacity for envelope proteins. Moreover, its sequence presented no homology to that of the HBV genome.¹⁻³

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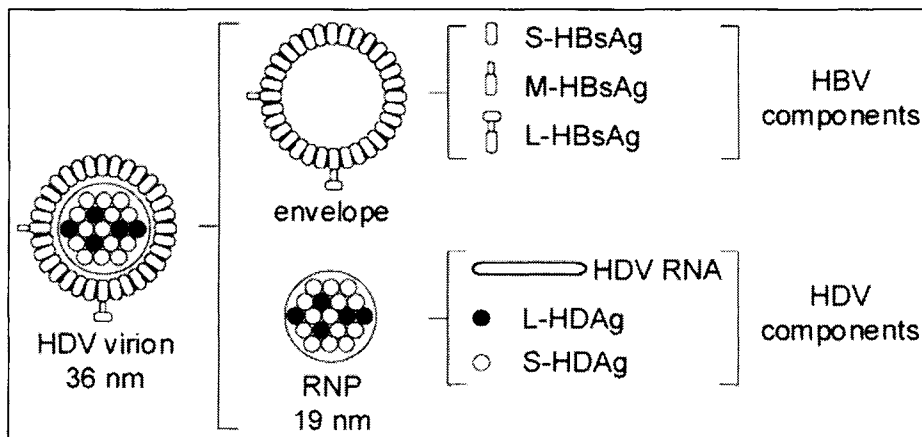


Figure 1. Schematic representation of the HDV particles found in an infectious serum. The virion comprises two types of elements: i) the viral envelope of HBV origin, including the HBV envelope proteins S-HBsAg, M-HBsAg and L-HBsAg, and ii) the ribonucleoprotein (RNP) that comprises the circular genomic RNA associated to multiple copies of the HDV-encoded delta proteins, S-HDAg and L-HDAg.

The Structure of the HDV Particle

The HDV virions are heterogeneous in size with an average diameter of 36 nm (Fig. 1). They display a chimeric structure: the protein content of the envelope consists of the HBV surface proteins, whereas the inner ribonucleoprotein (RNP) complex is made of HDV-specific elements. The HDV RNP is composed of a 1,700 nucleotides single-stranded genomic RNA associated to multiple copies of the HDV encoded delta protein, with a molar ratio of protein to RNA of approximately 200. The delta protein bears the hepatitis delta antigen (HDAg), and it appears under two isoforms: the small form (S-HDAg) is a 195-amino acid polypeptide; and the large form (L-HDAg) is 19 amino acids longer, and it arises as a consequence of a RNA editing event on a replication intermediate of the genome.³⁻⁵

HDV RNPs can be examined by electron microscopy after subjecting virions to a non-ionic detergent and a reducing agent. They appear as spherical, corelike structures, with no apparent icosahedral symmetry and a diameter of approximately 19 nm. The HDV envelope appears undistinguishable from that of HBV. It consists of a lipid membrane in which the three HBV coat proteins carrying the hepatitis B surface antigen (HBsAg), and designated small (S-HBsAg), middle (M-HBsAg) and large (L-HBsAg), are embedded. These proteins are present at a ratio estimated at 95/5/1, respectively, as opposed to 4/1/1 in the envelope of HBV virions (Dane particles).^{1,6}

Therefore HDV appears to be directly dependant on HBV for acquiring its envelope and, for that reason, it should be considered as a defective virus and a satellite of the latter. It does not fulfill the criteria for the definition of a virus; nonetheless, it is referred to as *Hepatitis delta virus* (HDV) for practical reason, and it constitutes the only species of the *Deltavirus* genus. In an infectious serum, HDV particles are present at high titer (up to 10^{11} particles per milliliter) along with different types of HBV particles: the infectious Dane particles and the empty sub-viral particles. They have a similar outer envelope consisting of the HBV coat proteins.^{1,6}

Are HBV Helper Functions Limited to Supplying Envelope Proteins to HDV?

Based on the structure of the HDV virion, it would appear that HBV is just a provider of coat proteins. This has been demonstrated experimentally: the transfection of mammalian cells with a cloned HDV cDNA, in the absence of HBV, leads to replication of the HDV RNA and formation of RNPs, demonstrating that HBV is not essential at this level of the HDV cycle. However, the absence of viral RNA or HDAg proteins in the culture medium of transfected cells indicates that progeny RNPs can not be released. Thus, unlike nonenveloped viruses, which are capable of inducing cell membrane lysis for particle release, and contrary to retroviral nucleocapsids that are capable of budding at the plasma membrane in the absence of envelope proteins, HDV cannot release its RNPs by lack of an export system. When cells are cotransfected with HDV cDNA and a plasmid driving the expression of the HBV envelope proteins (in the absence of HBV replication), the RNPs are released as enveloped virions. HBV assists HDV assembly and secretion by just providing the envelope proteins necessary to assemble export vesicles. Moreover, the L-HBsAg and M-HBsAg are not required for this process.⁷

Why Is HBV Best Suited for Assisting HDV?

HBV belongs to the *Hepadnaviridae* family. It is an enveloped virus that contains a circular partially double-stranded DNA, but it replicates its genome through a reverse transcription mechanism. Most important to the HDV-HBV interaction is the type of assembly mechanism that HBV has developed: budding occurs at the membrane of the intermediate compartment (IC), and is driven by the viral coat proteins. The three envelope proteins, S-HBsAg, M-HBsAg and L-HBsAg are found at the surface of HBV virions but in reality, S-HBsAg alone provides the driving force of the budding process. As a result, the vast majority of S-HBsAg proteins are secreted as empty subviral lipoprotein particles.⁶

HBV envelope proteins are coded by a single open reading frame on the HBV genome, using three different sites for initiation of translation. The S-HBsAg protein is 226 amino acid residues in length, and the M-HBsAg protein consists of the S polypeptide and 55 additional residues (the preS2 domain) at the N-terminus. L-HBsAg comprises the entire M polypeptide with an additional N-terminal polypeptide (referred to as the preS1 domain) of 108 residues (Fig. 2).

S-HBsAg is an integral protein, synthesized at the endoplasmic reticulum (ER) membrane (Fig. 3). It is anchored in the lipid bilayer through a N-terminal transmembrane domain (TMD1) between residues 4 and 28. It comprises: the TMD1; a downstream cytosolic loop (residues 29-79); a second TMD (TMD2) between residues 80 and 100; the antigenic loop (residues 101-164) that contains the immunodominant epitopes facing the ER lumen (or the surface of extracellular particles); and a hydrophobic C-terminus (residues 165-226), whose structure is predicted to contain two alpha-helices. M-HBsAg has a membrane topology similar to that of S-HBsAg, with its N-terminal preS2 facing the ER lumen. L-HBsAg, whose C-terminal half consists of the entire S domain, adopts two topologies at the ER membrane: its N-terminal preS domain (preS1 + preS2) is either luminal (external on secreted virions) or cytosolic (internal on secreted virions). The internal conformation is involved in recruiting the HBV nucleocapsid for virion assembly, and the external form corresponds to a receptor binding function necessary for viral entry (Fig. 2).⁶

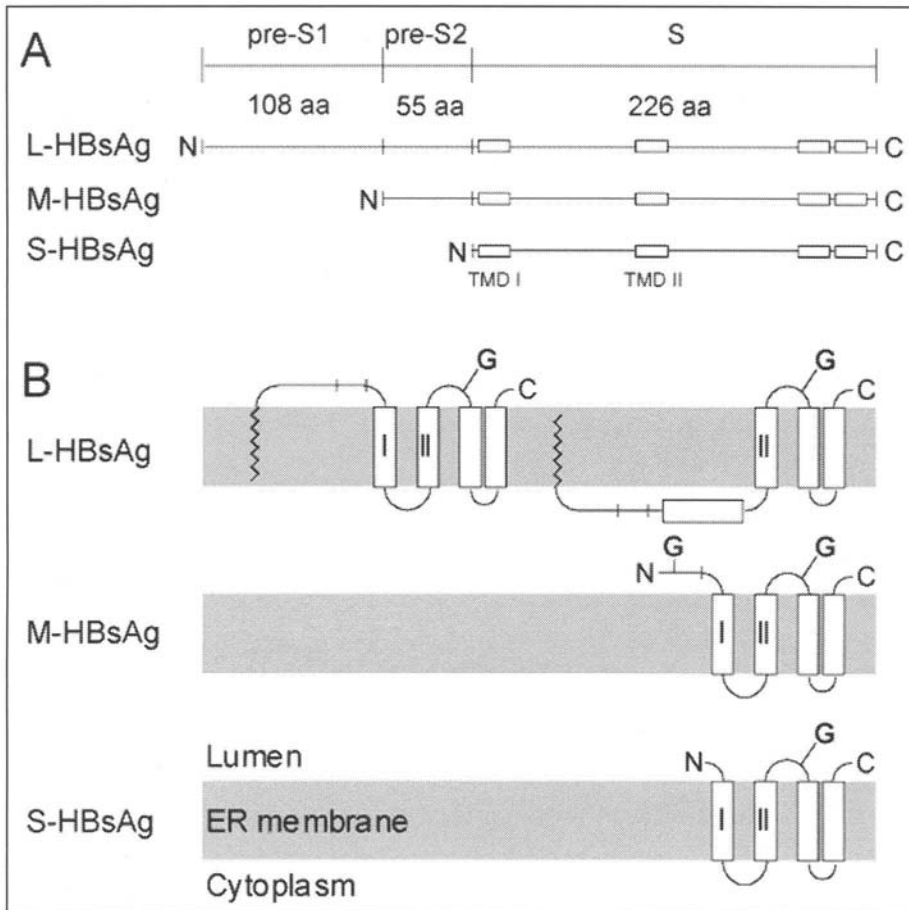


Figure 2. A) Domains of the HBV envelope proteins open reading frame (upper line). The L-HBsAg, M-HBsAg and S-HBsAg proteins are translated from three in-frame initiation sites located at the N-terminus of the preS1, preS2 and S domains, respectively. Open rectangles indicate experimentally defined TMDs, TMD I, TMD II. B) Membrane topology of the HBV envelope proteins. M-HBsAg and S-HBsAg adopt a similar topology at the ER membrane. The two transmembrane topologies of L-HBsAg are represented: the preS domain (preS1 + preS2) can reside on the cytoplasmic side of the ER membrane (right panel), or it can be translocated in the ER lumen (left panel). Broken line indicates the myristate group linked to the N-terminus of L-HBsAg. Grey rectangles represent the lipid ER membrane. Open rectangles represent TMDs. G, glycosylation site.

S-HBsAg proteins can dimerize at the ER membrane through lateral protein-protein interactions, and the resulting aggregates bud spontaneously into the lumen of the IC compartment (Fig. 4). This mechanism is very efficient and central to the maturation of HBV and HDV virions. It is only when L-HBsAg proteins are included in the S-HBsAg aggregates that HBV nucleocapsid can be incorporated in the budding process to assemble mature HBV virions. However this is scarcely observed in comparison with subviral particle formation. It is estimated that more than 99% of HBV-related particles are subviral, and this translates into an

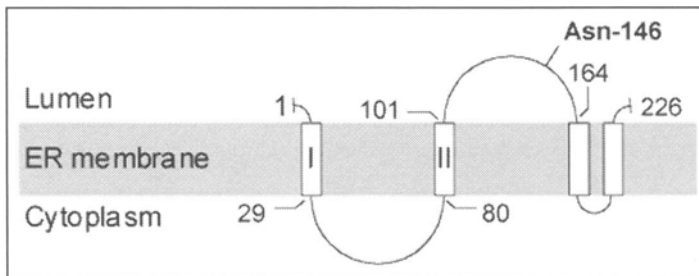


Figure 3. The small HBV envelope protein (S-HBsAg) is synthesized at the ER membrane. It comprises TMD1 (I), amino acid residues 4-28; a cytosolic domain, 29-79; TMD2 (II), 80-100; the antigenic loop, 101-164; two C-terminal TMDs, 165-226. The glycosylation site, Asn-146, in the antigenic loop is indicated.

average infectious serum containing approximately 10^{12} to 10^{13} subviral particles and only 10^8 HBV virions.⁶ Thus, HBV appears to have developed an overactive budding mechanism. It is carried out by the S-HBsAg protein itself, and it leads to the production of lipoprotein export vesicles that, for the vast majority, travel empty. It constitutes an export system at the disposal of coinfecting HDV.⁷

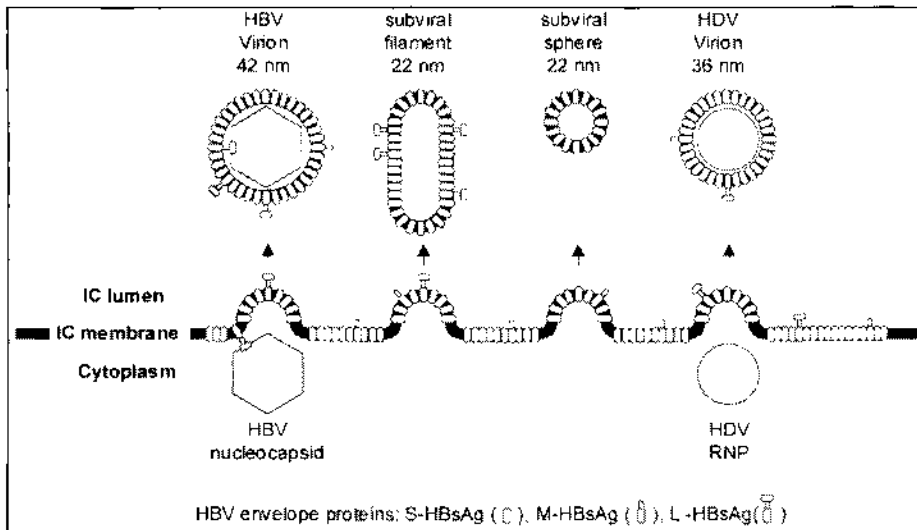


Figure 4. Model for budding of HBV and HDV particles into the lumen of the cellular intermediate compartment (IC). HBV envelope protein aggregates bud spontaneously at the IC membrane. When aggregates include S-HBsAg proteins only, or S-HBsAg + M-HBsAg, it leads to the formation of spherical subviral particles. When aggregates include L-HBsAg in the absence of HBV nucleocapsid, it leads to the secretion of filamentous subviral particles. When aggregates include L-HBsAg in the presence of HBV nucleocapsid, budding leads to the secretion of HBV virions (Dane particles). When HDV RNP is present, it can be included in the aggregates, irrespective of the presence of L-HBsAg. Incorporation of the L-HDAg protein in the HDV envelope confers infectivity.

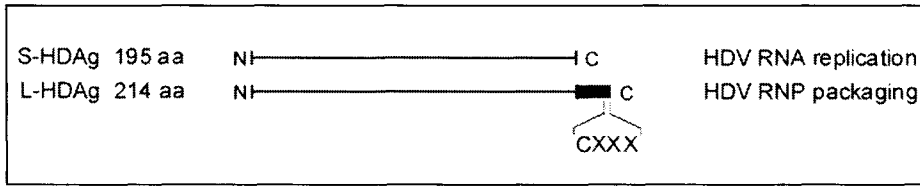


Figure 5. The small form of the delta protein (S-HDAg) is 195-amino acids in length, and the large form (L-HDAg) has a C-terminal extension of 19 amino acids (black rectangle). S-HDAg is required for HDV RNA replication, and L-HDAg is required for packaging of the RNP by the HBV envelope proteins. The signal for farnesylation, CXXX box (C = cysteine, X = any amino acid) is indicated.

How Do S-HBsAg and the RNP Interact with Each Other for HDV Assembly?

In general, virus assembly occurs at a specific site in the cell, and it requires the colocalization of the structural components. For most viruses, newly synthesized proteins are prevented from initiating budding reactions until all virion components are present at the site of assembly. This does not apply to HBV since budding can occur in the absence of HBV nucleocapsid, and this is to the advantage of HDV.

Overall, the formation of progeny HDV virions involves two processes: (i) assembly of the RNP and (ii) formation of the viral envelope. They are directed by two distinct viral species; they are independent of each other and spatially separated, occurring in the nucleus and at the IC membrane, respectively.

Therefore, a critical function that HDV must assume is the targeting of the RNP to the HBV budding system. HDV RNP is composed of the two forms of delta proteins: S-HDAg and L-HDAg (Fig. 5). Both proteins can interact with each other and the genomic RNA to assemble the HDV RNP complex. It is localized primarily in the nucleus, and it must travel to the cytoplasm for packaging with the envelope proteins. This is determined, in part, by the C-terminal 19 amino-acid polypeptide of L-HDAg, which contains a CXXX signal (where C = cysteine and X = any amino acid) for farnesylation.⁸ The farnesyl group probably serves to anchor the RNP in the ER membrane where the envelope proteins assemble, and, as expected, treatment of HDV producing cells with a farnesyl transferase inhibitor prevents assembly of RNPs into enveloped particles. When expressed with the HBV envelope proteins in the absence of HDV RNA and S-HDAg, L-HDAg protein can be packaged and secreted in the subviral particles (Fig. 6). The C-terminal 19 amino acids, including the CXXX box are likely to constitute the packaging signal on L-HDAg, since their appending to the C-terminus of a foreign protein, namely c-H-ras, leads to the cosecretion of the latter with HBV subviral particles. Yet, for HDV maturation, it is unclear whether L-HDAg binds to S-HBsAg during budding or beforehand, and whether the free form of L-HDAg associates with the envelope in addition to the RNP-associated form.²

Regarding the role of the S-HBV protein, it is worth noting that the member of the *Hepadnaviridae* family closest to HBV, the *Woodchuck hepatitis virus* (WHV), can assist HDV propagation because its S envelope protein (S-WHsAg) is competent for HDV RNP envelopment; whereas the envelope protein of a more distantly related *Hepadnaviridae*, namely the *Duck hepatitis B virus* (DHBV), is unable to fulfill this function. Thus determinants that are specific for HDV maturation on the S-HBsAg protein should be present on S-WHsAg and absent on the small DHBV envelope protein (S-DHBsAg). When compared to S-HBsAg or

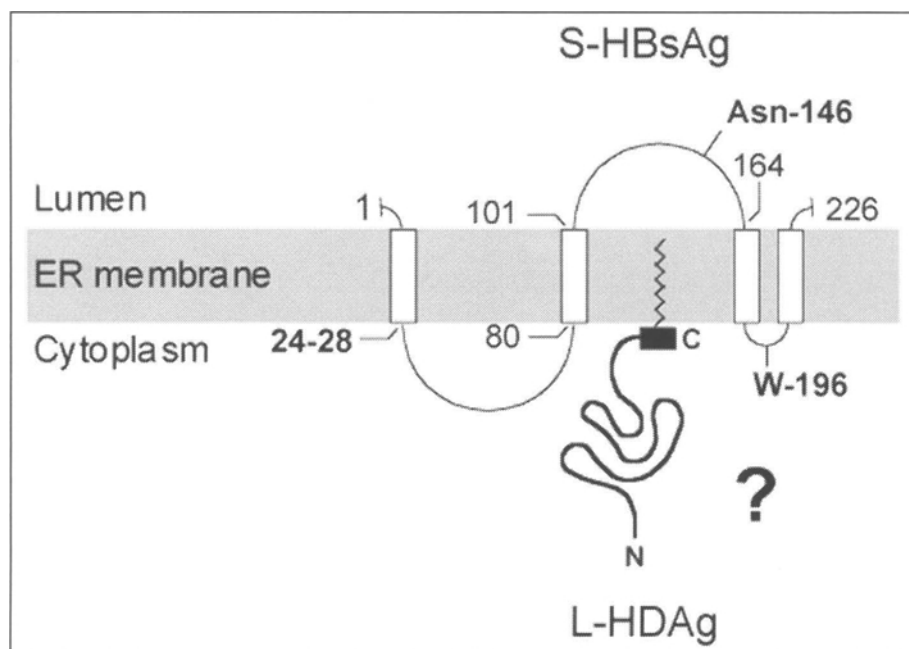


Figure 6. The small HBV envelope protein (S-HBsAg) and the large hepatitis delta protein (L-HDAg) have a crucial role in HDV assembly. They colocalize at the ER or IC membrane and probably interact with each other. The localization of L-HDAg at the ER membrane is thought to depend on the farnesyl group attached to its C-terminus (broken line). Black rectangle indicates the C-terminal packaging signal of L-HDAg. Amino acids of the S-HBsAg protein that are important for envelopment of the HDV RNP are indicated in bold.

S-WHsAg, the S-DHBsAg polypeptide appears to lack the region corresponding to the antigenic-loop (Fig. 3). Indeed, when part of this domain (from residues 107 to 147) is experimentally deleted on S-HBsAg, it leads to a reduction in the capacity of the mutant for HDV maturation.⁹ Interestingly, the same mutant was fully competent for packaging HDAg-L proteins, suggesting that the hindrance observed in RNP envelopment may reflect a reduced flexibility of the envelope, which could no longer accommodate an RNP, rather than a lack of binding to the RNP. The deficiency in HDV maturation observed with the antigenic-loop-deleted S-HBsAg, or with S-DHBsAg, could also be explained, at least in part, by the absence of a glycosylation site, since the removal of Asn-146 on S-HBsAg was shown to prevent glycosylation and to inhibit HDV particle formation.¹⁰

The cytosolic loop of S-HBsAg (residues 28 to 80) has a topology at the ER membrane that is prone to interact with the HDV RNP during virion assembly. In fact, a genetic analysis revealed the importance of residues 24-28 in HDV maturation, but the contribution of this sequence to a direct binding to the RNP was not demonstrated. The same study also revealed that sequences 28-47 and 49-59 do not contain any motif essential for HDV morphogenesis. It is worth noting that the very same region of the cytosolic loop (residues 33 to 59) contains motifs essential for HBV nucleocapsid envelopment.

When the C-terminus of S-HBsAg was examined, it was found that the tryptophane residue at position 196 was important for HDV assembly. This region (164-226) is highly hydrophobic and predicted to contain two TMDs located at positions 173-193 and 202-222. They bracket a short sequence (194-201), including Trp-196, that presents a low degree of flexibility. Hydrophobicity and secondary structure predictions are compatible with the orientation of Trp-196 at the cytosolic side of the ER membrane in a position potentially adequate for interaction with the RNP.

Two observations that bear on the RNP envelopment mechanism, can be made. First, HDV assembly was not completely abolished when residues 24-28, Asn-146 or Trp-196 were singly deleted on S-HBsAg, suggesting that direct interactions, if any, between HDV RNP and S-HBsAg may involve several domains or residues distributed on the entire polypeptide. They could adopt a spatial proximity conferred by the complex organization of the polypeptide at the ER membrane. Second, the fact that motifs identified as essential to HDV assembly, such as residues 24-28 or Trp-196, are dispensable for subviral particle secretion and yet strictly conserved among HBV and WHV isolates, suggests that the selection pressure that has led to their conservation concerns functions other than those involved in subviral particle assembly. Thus, HDV RNP envelopment may rely upon S-HBsAg residues that are operative in maturation or infectivity of HBV virions. But this remains to be proven experimentally.¹¹

For a better understanding of the HDV maturation process, the determinants of incorporation of L-HDAg proteins into the subviral particles need to be sorted from those involved in RNP envelopment. In the former case, assembly should proceed through colocalization of the particle components and a specific interaction between L-HDAg and S-HBsAg; whereas in the latter case, assembly is likely to depend also on the constraints exerted on the envelope to accommodate a 19 nm RNP. The fact that HBV manages to assemble three types of particles, namely the 22 nm subviral spheres, the filamentous forms and the 42 nm Dane particles, indicates that S-HBsAg can modulate its intrinsic membrane bending force. Therefore, the flexibility of the HBV envelope protein is another important characteristic of this virus (Fig. 4).

But is there an actual need for a direct interaction between S-HBsAg and L-HDAg, or could HDV RNPs be passively incorporated in the HBV budding vesicles? In the light of a recent study that measured the concentration of HDAg proteins at up to six millions copies per infected cell, it seems that the encounter between L-HDAg and S-HBsAg, or any cellular factor involved in this process, should be facilitated.⁴ It would thus be interesting to test the capacity of other viruses such as *Coronaviruses* or *Spumaviruses* to substitute for HBV in HDV assembly. For both viruses, as for HBV, budding occurs at the post-ER/preGolgi membrane, and it is driven by the viral coat proteins.

However, one could argue for the requirement of a specific interaction between S-HBsAg and HDV RNPs based on the following observations: (i) sera collected at the peak of an acute HDV infection usually contain a high proportion of HDV virions to empty subviral particles, (ii) although produced less abundantly than S-HBsAg, S-DHBsAg proteins expressed in human cells are not capable of HDV maturation, (iii) a direct protein-protein interaction between S-HBsAg and L-HDAg has been reported using a far-Western binding assay, and (iv) synthetic peptides specific for HBV envelope proteins have been shown to bind both L-HDAg and S-HBsAg proteins.¹⁻³

The Infectivity of the HDV Virions

For propagation, secreted virions must be redirected to a non infected cell. Therefore, it was expected for the L-HBsAg protein, which mediates HBV infectivity, to be required as an integral component of the HDV envelope. This was demonstrated in an *in vitro* culture system: HDV particles coated with the S-HBsAg protein, or S-HBsAg and M-HBsAg, were not infectious when tested on primary hepatocyte cultures; but when L-HBsAg was coexpressed with S-HBsAg, infectivity was restored.¹²

In an HBV-infected cell, the incorporation of L-HBsAg proteins in budding vesicles has the following consequences: (i) it makes the budding unit competent for HBV nucleocapsid envelopment; (ii) it exerts a partial retention of subviral particle secretion; and (iii) it induces a change in the shape of the empty particles (from spherical to filamentous). It is not clear if these functions are fully operative or partially altered when the HDV RNPs are present (Fig. 4).⁶

Overall, the HDV life cycle depends on only two HBV elements: the S-HBsAg protein for the export of the RNP, and the L-HBsAg protein for entry in a noninfected hepatocyte. As for the M-HBsAg protein, its role needs L-HBsAg to envelope, is not essential for assembly or *in vitro* infectivity of HDV. But contrary to HBV that its nucleocapsid only in the presence of L-HBsAg, HDV can be coated quite efficiently with an envelope devoid of L-HBsAg. And it is likely that most HDV particles present in an infectious serum are coated with S-HBsAg proteins only (or S-HBsAg and M-HBsAg), and that only a minority contain L-HBsAg. The inclusion of L-HBsAg in the HDV envelope is thought to occur through lateral protein-protein interactions that are established between L-HBsAg and S-HBsAg at the ER membrane before budding, but the proportion of L-HBsAg proteins that are needed in the envelope for infectivity is unknown. With regard to virus entry into the host cell, it seems reasonable to assume that HBV and HDV use the same cellular receptors on the human hepatocyte, but the identities of the receptors remain unknown. At the post-binding steps, internalization of an HDV RNP or that of an HBV nucleocapsid, most likely follows a specific pathway to which S-HBsAg and L-HBsAg may participate.^{1,6,12}

What Are the Effects of HDV Infection on the HBV Life Cycle?

Since HDV is directly dependent on HBV for propagation, it can be transmitted concomitantly with HBV to an individual who has no history of prior HBV infection—this is referred to as the coinfection pattern—or it can be transmitted to an HBV chronic carrier—this is referred to as the superinfection. Coinfections are often acute and self-limited, and they are characterized by a concomitant replication of both HBV and HDV; whereas superinfections often cause severe acute hepatitis and chronic type D hepatitis in 70% of the cases.¹ They also lead to the inhibition of HBV replication during the acute phase of HDV infection. This phenomenon has been described in both humans and the experimentally infected chimpanzee, but it remains poorly understood. It could result from a direct suppression of HBV replication exerted by the coexpressed HDV proteins, RNA or RNPs, or it could be the consequence of an indirect interfering mechanism which may involve inflammatory cytokines.¹

There are at least three HDV genotypes with different geographic distributions and a sequence divergence as high as 38% at the nucleotide level. Genotype I is ubiquitous, genotype II has been found primarily in east Asia, and genotype III only in South America. Eight HBV genotypes (designated A to H) have been described, presenting also different geographic distributions. The heterogeneity of the disease pattern caused by HDV-HBV infections, which has been observed worldwide, may depend on specific HDV on HBV genotypes. The most severe form has been recorded in South America where a genotype III HDV was associated to a genotype F HBV.¹

Conclusion

Among enveloped viruses that achieve maturation through a nucleocapsid-independent assembly mechanism, HBV has developed the most active budding process. It is carried out by the S-HBsAg envelope protein itself, and it leads to the formation of a large excess of empty subviral particles over mature virions. For that reason, HBV appears the best-suited virus for assisting a coatless virus such as HDV to export its genome from an infected cell by supplying the transport vesicles. Though not proven, a specific interaction between the HDV RNP and S-HBsAg is probably needed to ensure an efficient virion assembly. Recent experiments based on genetic analysis have contributed to the understanding of the intracellular HDV-HBV interactions involved in HDV maturation, but it is expected that further progress in this direction will be achieved when ultrastructural data for both the HBV envelope and the HDV RNP become available.

References

1. Gerin JL, L CJ, Purcell RH. Hepatitis Delta Virus. In: Knipe DM, Howley PM, eds. *Fields Virology*. Philadelphia, PA: Lippincott Williams & Wilkins, 2001:3037-3050.
2. Taylor JM. Replication of human hepatitis delta virus: Recent developments. *Trends Microbiol* Apr 2003; 11(4):185-190.
3. Lai MM. The molecular biology of hepatitis delta virus. *Annu Rev Biochem* 1995; 64:259-286.
4. Gudima S, Chang J, Moraleda G et al. Parameters of human hepatitis delta virus genome replication: The quantity, quality, and intracellular distribution of viral proteins and RNA. *J Virol* 2002; 76(8):3709-3719.
5. Rizzetto M, Hoyer B, Canese MG et al. Delta agent: Association of delta antigen with hepatitis B surface antigen and RNA in serum of delta-infected chimpanzees. *Proc Natl Acad Sci USA* 1980; 77(10):6124-6128.
6. Ganem D, Schneider R. Hepadnaviridae: The viruses and their replication. In: Knipe DM, Howley PM, eds. *Fields Virology*. Philadelphia, PA: Lippincott Williams & Wilkins, 2001:2923-2969.
7. Wang CJ, Chen PJ, Wu JC et al. Small-form hepatitis B surface antigen is sufficient to help in the assembly of hepatitis delta virus-like particles. *J Virol* 1991; 65(12):6630-6636.
8. Glenn JS, Watson JA, Havel CM et al. Identification of a prenylation site in delta virus large antigen. *Science* 1992; 256(5061):1331-1333.
9. O'Malley B, Lazinski D. A hepatitis B surface antigen mutant that lacks the antigenic loop region can self-assemble and interact with the large hepatitis delta antigen. *J Virol* Oct 2002; 76(19):10060-10063.
10. Wang CJ, Sung SY, Chen DS et al. N-linked glycosylation of hepatitis B surface antigens is involved but not essential in the assembly of hepatitis delta virus. *Virology* 1996; 220(1):28-36.
11. Jenna S, Sureau C. Mutations in the carboxyl-terminal domain of the small hepatitis B virus envelope protein impair the assembly of hepatitis delta virus particles. *J Virol* 1999; 73(4):3351-3358.
12. Sureau C, Guerra B, Lanford RE. Role of the large hepatitis B virus envelope protein in infectivity of the hepatitis delta virion. *J Virol* 1993; 67(1):366-372.