African Journal of Biotechnology Vol. 7 (25), pp. 4911-4916, 29 December, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

*Review*

# Structure and replication of hepatitis delta virus

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Accepted 8 December, 2008

**Hepatitis delta virus is the causative agent of one of the most severe forms of human virus hepatitis. It is a small and simple pathogen whose genome consists of a single RNA molecule of 1.7 Kb that encodes for only one antigen. The virus almost completely relies on the host cell machinery for replication and propagation of infection. Despite its simplicity, several aspects of virus replication and pathogenesis still remain uncovered. An overview of virus structure and replication mechanisms as well as of its interaction with the hepatitis B virus is performed in this review.**

**Key words:** Hepatitis D virus, delta antigens, RNA replication.

## THE VIRION

Hepatitis delta virus (HDV) is the smallest human pathogen so far identified. HDV is considered to be a satellite virus of hepatitis B virus (HBV), and is the only member of a relatively recent, new Deltavirus genus (Taylor, 1996).

The virion is a spherical particle of 36 - 43 nm diameter (He et al., 1989). This particle consists of a short negative single stranded circular RNA of about 1.7 kb, the virus genome, small and large virus encoded delta antigens (S-HDAg and L-HDAg, respectively), and hepatitis B surface antigens (HBsAgs). The HDV nucleocapsid consists of a ribonucleoprotein (RNP) complex that includes a single RNA molecule bound to about 70 molecules of both delta antigens (Ryu et al., 1993). This nucleocapsid has a diameter of about 19 nm, and is surrounded by an outer envelope consisting exclusively of HBsAgs and host cell lipids (Tiollais et al., 1985). All the three forms of HBsAgs, large, middle, and small (L, M, S) can be found in HDV infectious particles (Tiollais et al., 1985). The HDV particles predominantly contain the S-HBsAg, which is sufficient for virion maturation (Sureau et al., 1993) but the L-HBsAg was also reported to be required for assembly of HDV virions. The role of the M antigen is not fully understood, but it is thought that it may play a minor role in virion assembly. In HDV the relative ratios of L: M: S has been reported as 1:5:95 (Bonino et al., 1996).

## THE VIRUS RNA

The HDV RNA genome has unique features among animal viruses. It consists of a circular, single-stranded, RNA molecule of negative polarity, and about 1,700 nucleotides. This RNA displays a high degree of internal base-pairing, about 70% of the whole molecule, resulting in a rod shaped secondary structure similar to that found in plant viroids (Figure 1). One single open reading frame (ORF) was identified in the complementary virus RNA, also called the antigenome. This ORF is located between nucleotides 1014 and 1598, and was found to be transcribed into a 0.8 Kb mRNA which encodes for the S-HDAg (Macnaughton et al., 2002; Moraleda and Taylor, 2001). During replication, however, this ORF can be extended by 57 nucleotides as a result of deamination reaction that occurs at the adenine residue on position 1015 of an amber stop codon UAG, transforming it onto a triptophan codon UGG (Casey and Gerin, 1995). The enzyme responsible for this editing mechanism was identified as being the small form of cellular adenosine deaminase (ADAR1; Casey and Gerin, 1995). Another important feature of the virus genomic and antigenomic RNA molecules is the 85 nucleotide ribozyme domain (Perrotta and Been, 1991; Rosenstein and Been, 1991). This activity is essential during replication in order to catalyse the precise self-cleavage of multimeric RNA molecules into unit-length linear (Sharmeen et al., 1989) fragments that subsequently self-ligate to form circles. The HDV ribozymes normally function as part of the larger genomic and antigenomic RNA. The sequence of both genomic and antigenomic ribozymes can be folded into a similar overall secondary structure consisting of base-paired elements, joining sequences and hairpin loops.

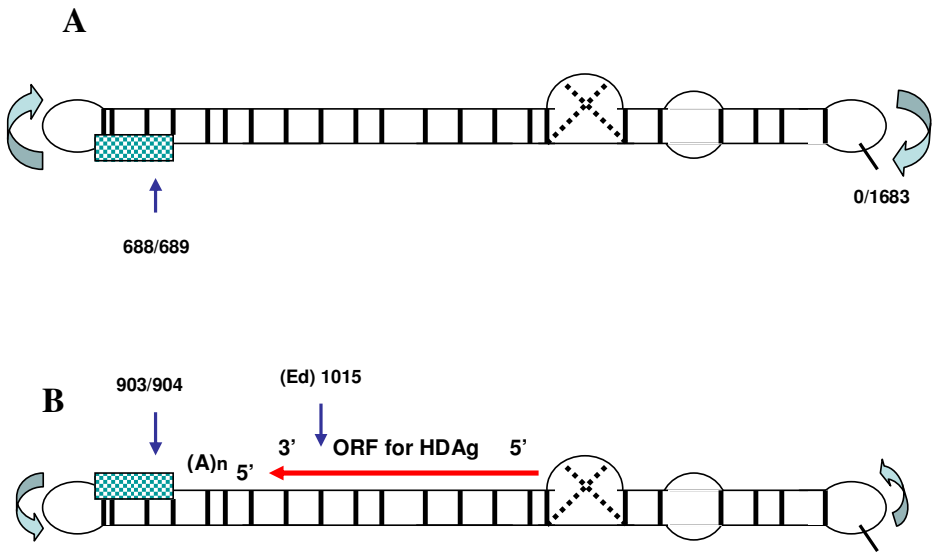


**Figure 1.** Representation of the rod-shaped structure of the HDV genomic (A) and antigenomic (B) RNAs. The green box represents the ribozyme domain. The cleavage site is indicated by an arrow. In the antigenomic RNA, the editing site in the amber stop codon UAG is also indicated.

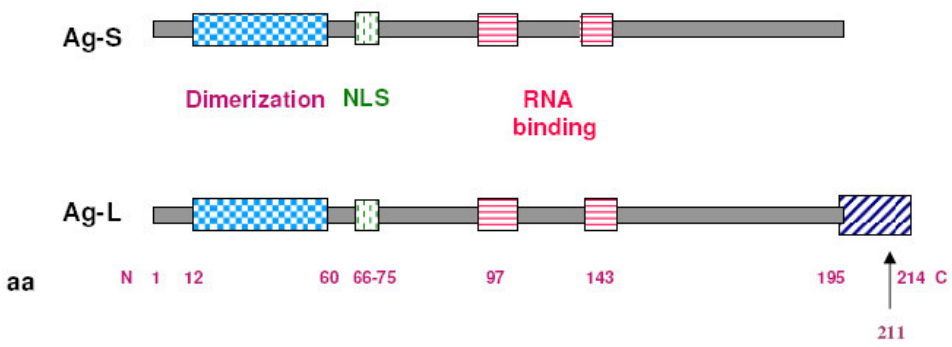


**Figure 2.** Functional domains of the delta antigens. The dimerization domain, the nuclear localization signal, and the RNA binding domains are represented by boxes. The cysteine site for isoprenylation is indicated by an arrow.

It was estimated that inside an infected liver cell the number of copies of each of the HDV derived RNAs is about 300,000 for genomic molecules, 50,000 for antigenomic, and 600 for the corresponding mRNA (Chen et al., 1986). The HDV mRNA was found to be polyadenylated and capped (Hsieh and Taylor, 1991; Nie et al., 2004). These post-transcriptional modifications are common to all cellular RNA polymerase II synthesized mRNAs, pointing to a possible role of this enzyme in HDV RNA replication.

## THE DELTA ANTIGENS

As mentioned above, two forms of the delta antigen, small and large, are found in HDV infectious particles and in infected liver cells. These two forms are derived by the same ORF as a result of an editing mechanism that occurs during replication. The small form is a 195 aa protein of about 24 KD which is synthesised early in the replication cycle. The large delta antigen is a 27 KD protein similar to the S-HDAg, with the exception of the additional last C terminal 19 aa. As a consequence, both S-HDAg and L-HDAg share a number of common functional domains (Figure 2): a RNA binding domain, a dimerization domain (Lazinski and Taylor, 1993), and a nuclear localization signal (Alves et al., 2008). However, these antigens play different roles in virus replication; S-HDAg stimulates RNA replication (Yamaguchi et al., 2001; Cheng et al., 2003) and positively regulates ribozyme activity (Jeng et al., 1996), while L-HDAg seems to

repress it in *trans* and is required for virion maturation and interaction with HBsAgs at later stages in the replication cycle (Chang et al., 1991; Glenn et al., 1992).

The dimerization domain is coiled-coil structure located at aminoacids 12 - 60. This dimerization, or even multimerization, event is important for L-HDAg to interact with S-HDAg and act as a dominant negative inhibitor of replication. Mutations that eliminate or alter the coiled-coil structure either greatly reduce or totally eliminate the ability of S-HDAg to function as a *trans* activator of replication. These same mutations also prevent L-HDAg from inhibiting RNA replication and inhibit its function in mediating the co-packaging of S-HDAg (Chang et al., 1993, 1994). The nuclear localization signal consists of a single stretch of 10 aminoacids, EGAPPAKRAR, located in positions 66 - 75 (Alves et al., 2008). It is thought that the main function of this domain is to promote the nuclear import of HDV RNPs during the early stages of infection (Chou et al., 1998). The third shared domain is a bipartide RNA binding domain, localized between aminoacids 97 - 143 (Lazinski and Taylor, 1993). This domain consists of two stretches of arginine-rich sequences similar to arginine-rich motifs (ARMs) present in several other RNA binding proteins, such as rev and tat of human immunodefficiency virus. Both ARMs are required for the binding of HDAg to HDV RNA. This binding seems to be specific for both genomic and antigenomic HDV RNA strands.

The C-terminal 19 aminoacids domain of L-HDAg are unique to this protein. The fourth aminoacid from the C-terminus is a cysteine residue that constitutes an isoprenylation motif, and is prenylated with a geranyl-geranyl-prenoid (Glenn et al., 1992; Lee et al., 1994). The prenylation probably allows the L-HDAg to acquire the ability to interact with the envelope proteins of HBV, playing an important role in virus assembly.

A number of post-translational modifications have already been described for both forms of the delta antigens. They are both phosphorylated *in vivo*, although the large form is six times more heavily phosphorylated than the small form (Chang et al. 1988; Hwang et al., 1992). The occurrence of putative phosphorylation sites in HDAg for casein kinase II and protein kinase C suggests that HDAgs are substrates for these protein kinases (Yeh et al., 1996). In fact, both kinases were shown to positively modulate the HDV RNA replication (Yeh et al., 1996). Furthermore, PKC was found to be the kinase that associated with and subsequently phosphorylated S-HDAg. Phosphoaminoacid analysis indicated that L-HDAg was phosphorylated at serine residues and S-HDAg was phosphorylated at both serine and threonine rsidues (Chang et al., 1988; Mu et al., 1999). In addition, other post-translational modifications were described for HDAgs, namely methylation and acetylation. Phosphorylation of serine residue 123 (Tan et al., 2004), methylation of arginine residue 13 (Mu et al., 2004), and acetylation of lysine residue 72 (Li et al., 2004) were observed to affect the subcellular localization of HDAgs and to be important for antigenomic RNA replication.

## THE REPLICATION CYCLE

HDV replicates exclusively in liver cells. The HDV envelope contains HBV surface antigens and it is possible both viruses depend on the same host receptor for entry into the cell. It has been established that sequences within the preS1 region of the L form of the HBsAg are essential for attachment to a specific cell surface receptor. However, this receptor has not yet been identified. Moreover, the subsequent steps of virus entry and uncoating still remain to be uncovered.

HDV replication is totally independent of any HBV sequence or function. Upon entry into the cell, HDV RNPs are transported into the nucleus where replication of the HDV genome takes place. This transport seems to be mediated by the NLS present on the HDAgs but the cellular transport receptors involved in this pathway are unknown. In support of this interpretation, the delta proteins and the genomic and antigenomic RNAs do seem to be predominantly localised in the nucleus (Cunha et al., 1998; Bell et al., 2000). L-HDAg and antigenomic RNA are co-localised in discrete nuclear substructures, while genomic RNA strands and a substantial amount of S-HDAg are distributed more broadly (Bell et al., 2000). The accumulation of specific HDV components in discrete foci in the nucleus is not probably relevant for virus replication. In fact, these substructures are not sites of active RNA synthesis, and do not colocalize with several components of the cellular transcription and processing machinery (Cunha et al., 1998).

Once entering the nucleus of liver cells, the HDV RNP is thought to initiate replication by a rolling circle mechanism, similar to that found in plant viroids, leading to the synthesis of multimeric antigenomic RNA molecules (Figure 3) (Gerin et al., 2001). The laters are then self cleaved at precise monomeric intervals and religated into antigenomic circles which serve as templates for synthesis of genomic RNA molecules by a similar mechanism. During the HDV replication cycle, several genomic length RNA species are produced: the genomic RNA encoding S-HDAg, the genomic RNA encoding L-HDAg, the antigenomic RNA enconding S-HDAg, and the antigenomic RNA encoding L-HDAg. The ratio of these species inside infected cells is estimated to be 59: 41: 9.7: 0.3 (Sheu, 2002). L-HDAg encoding RNA species are produced as a result of an editing mechanism, catalysed by host ADAR1, that occurs in the antigenome. There are evidences that intitiation of HDV replication may require *de novo* synthesised, intact S-HDAg (Dingle et al., 1998).

In the nucleus, HDV RNA is replicated by a mechanism of RNA-directed RNA synthesis. Since both forms of the delta antigens are to small to have polymerase activity, a

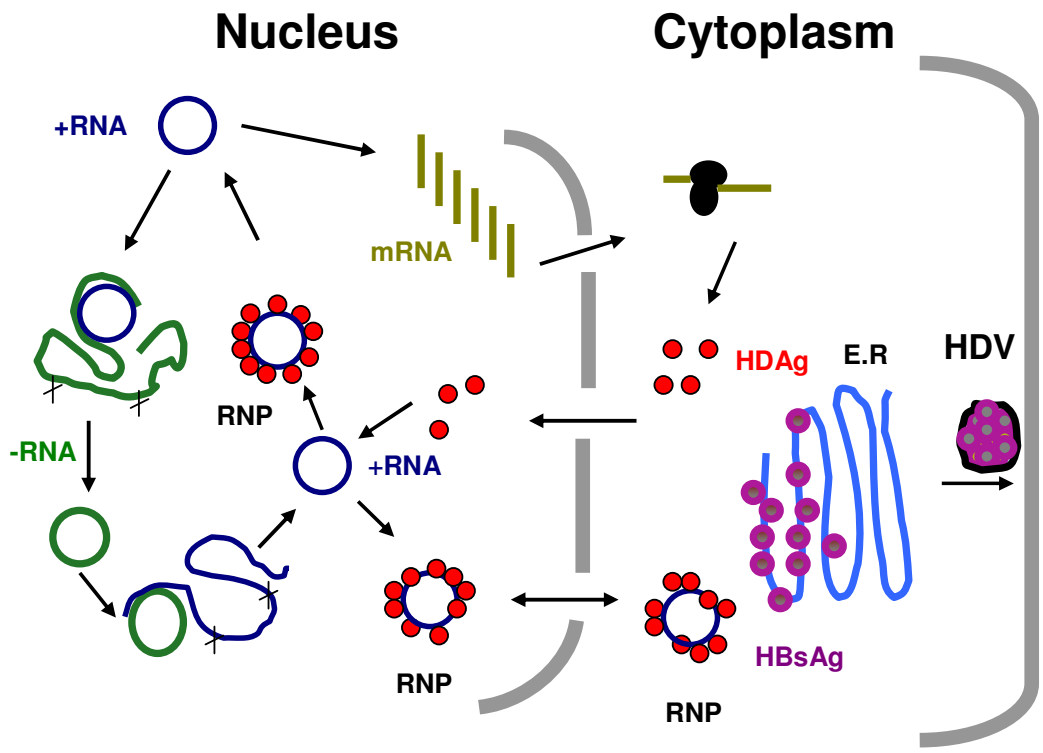


**Figure 3.** The HDV replication cycle. Inside the nucleus, the virus negative RNA genome is replicated by a rolling circle mechanism resulting in the production of multimeric antigenomes which are self-cleaved and religated. The positive RNA antigenomes serve then as template for synthesis of genomic RNA molecules by a similar mechanism as well as templates for transcription of mRNA. As HDAgs are synthesised in the cytoplasm they are recruited to the nucleus to form RNPs that subsequently are exported to the cytoplasm. Here, the RNPs interact with HBsAgs, mature HDV virions are assembled, and released from the cell by exocytosis (see text for details).

host RNA polymerase must therefore be involved in this process.

Several approaches have been used in attempts to identify the host RNA polymerase or polymerases involved in HDV RNA-directed transcription. One approach is the application of RNA polymerase inhibitors, namely α-amanitin, to cells in which HDV genome replication has been initiated. Low doses of α-amanitin have been observed to inhibit accumulation of both HDV mRNA and unit-length genomic species. In contrast, accumulation of antigenomic species was found to be resistant to high doses of α-amanitin, leading to the conclusion that transcription of the antigenome involves a polymerase other than RNA polymerase II (Modahl et al., 2000; Macnaughton et al., 2002). Additionally, making use of nuclear run-on assays in the presence of low doses of α-amanitin, Macnaughton et al. (2002) concluded that synthesis of genomic HDV RNA involves RNA polymerase II. Another approach consisted of the use of purified RNA polymerase II or nuclear extracts capable of DNA-directed transcription, and to add to these HDV RNA species as templates. The authors have used linear HDV RNA species and have detected what can be interpreted as short 3'-end addition of nucleotides to these templates (Gudima et al., 2000; Filipovska et al., 2000)). Using this same assay, it was also reported that the presence of the S-HDAg could act to increase the length of end additions (Yamaguchi et al., 2001, 2002). In conclusion, RNA polymerase II is very probably involved in HDV RNA synthesis but the involvement of other RNA polymerases can not be ruled out. Furthermore, no clear evidence has emerged regarding what might be a promoter element for HDV transcription.

Several studies tried to demonstrate the dependence between HDV replication and the presence of S-HDAg. *In vivo*, S-HDAg is known to stabilize HDV RNA circles and facilitate ribozyme cleavage (Lazinski and Taylor, 1993; Jeng et al., 1996). It is possible to speculate that this viral protein interacts with more cellular factors to promote HDV replication. One of these proteins is DIPA – delta interacting protein A. DIPA is a 202 aminoacid protein found to be 24% identical in sequence to HDAg (Brazas and Ganem, 1996). When overexpressed, this protein markedly inhibits HDV replication. More recently, the polypirimidine tract-binding protein-associated splicing factor (PSF; Greco-Stewart et al., 2006) was also reported to bind to HDV RNA.

Another protein that interacts with HDAg is the nuclear

phosphoprotein B23. It is present in abundance in both tumor cells and proliferating cells (Peter et al., 1990; Feuerstein et al., 1988). B23 has the ability to shuttle between the nucleus and the cytoplasm (Borer et al., 1989), binds the nuclear localization signal containing peptides (Szebeni et al., 1995) and thus serve as a shuttle protein in the nuclear import. B23 can interact with the NLS of both S-HDAg and L-HDAg suggesting that it may promote the nuclear import of HDAgs. It was earlier reported that HDV RNPs shuttle between the nucleus and the cytoplasm (Tavanez et al., 2002). While the nuclear import of HDV RNPs is mediated by the NLS present in HDAgs, the export to the cytoplasm seems to rather involve the interaction of specific sequences in the virus RNA with cellular export factors (Tavanez et al., 2002). Export of HDV RNPs to the cytoplasm is an important step in HDV replication. In the cytoplasm newly synthesized virus RNPs interact with the endoplasmic reticulum associated HBsAgs to form mature HDV virions which subsequently release from the cell by exocytosis. However, both the nuclear import and export pathways of HDV RNPs still need to be identified.

In our laboratory, we used a yeast two-hybrid assay to search for S-HDAg interacting eukaryotic proteins. Ten proteins were identified and confirmed to specifically bind *in vitro* to the S-HDAg. Work is now in progress in order to investigate the *in vivo* interaction of these proteins and their possible role in HDV replication.

## ACKNOWLEDGEMENTS

Work in the author's laboratory is financed by Fundação para a Ciência e Tecnologia (POCI/SAU-IMI/55112/2004).

### REFERENCES

Alves C, Freitas N, Cunha C (2008). Characterization of the nuclear localization signal of the hepatitis delta virus antigen. Virology 370: 12-21.

Bell P, Brazas R, Ganem D, Maul GG (2000). Hepatitis delta virus replication generatescomplexes of large hepatitis delta antigen and antigenomic RNA that affiliate with and alter nuclear domain 10. J. Virol. 74: 5329-5336.

Bonino F, Heermann KH, Rizzetto M, Gerlich WH (1996). Hepatitis delta virus: protein composition of delta antigen and its hepatitis B virus-derived envelope. J. Virol. 58: 945-950.

Borer RA, Lehner CF, Eppenberger HM, Nigg EA (1989). Major nucleolar proteins shuttle between nucleus and cytoplasm. Cell, 56 (3): 379-390.

Brazas R, Ganem D (1996). A cellular homolog of hepatitis delta antigen: implications for viral replication and evolution. Science. 294(5550): 2376-2378.

Casey JL, Gerin JL (1995). Hepatitis delta virus RNA editing: Specific modification of adenosine in the antigenomic RNA. J. Virol. 69: 7593-7600.

Chang MF, Baker SC, Soe LH, Kamahora T, Keck JG, Makino S, Govindarajan S, Lai MM (1988). Human hepatitis delta antigen is a nuclear phosphoprotein with RNA-binding activity. J. Virol. 62(7): 2403-2410.

Chang FL, Chen PJ, Tu SJ, Chiu MN, Wang CJ, Chen DS (1991). The large form of hepatitis δ antigen is crucial for the assembly of hepatitis δ virus. Proc. Natl. Acad. Sci. USA. 88: 8490-8494.

Chang MF, Sun CY, Chen CJ, Chang SC (1993). Functional motifs of delta antigen essential for RNA binding and replication of hepatitis delta virus. J. Virol. 67: 2529-2536.

Chang MF, Chen CJ, Chang SC (1994). Mutational analysis of delta antigen: effect on assembly and replication of hepatitis delta virus. J. Virol. 68: 646-653.

Chen PJ, Kalpana G, Gidberg J, Mason W, Werner B, Gerin J, Taylor J (1986). Structure and replication of the genome of hepatitis d virus. Proc. Natl. Acad. Sci. USA, 83: 8774-8778.

Cheng Q, Jayan GC, Casey JL (2003). Differential inhibition of RNA editing in hepatitis delta virus genotype III by the short and long forms of hepatitis delta antigen. J. Virol. 77: 7786-7795.

Chou HC, Hsieh TY, Sheu GT, Lai MM (1998). Hepatitis delta antigen mediates the nuclear import of hepatitis delta virus RNA. J Virol. 72(5): 3684-3690.

Cunha C, Monjardino J, Chang D, Krause S, Carmo-Fonseca M (1998). Localization of hepatitis delta virus RNA in human cells. RNA, 4: 680-693.

Dingle K, Bichko V, Zuccola H, Hogle J, Taylor J (1998). Intiation of hepatitis delta virus genome replication. J. Virol. 72: 4783-4788.

Feuerstein N, Spiegel S, Mond JJ (1988). The nuclear matrix protein, numatrin (B23), is associated with growth factor-induced mitogenesis in Swiss 3T3 fibroblasts and with T lymphocyte proliferation stimulated by lectins and anti-T cell antigen receptor antibody. J. Cell Biol. 107(5): 1629-1642.

Filipovska J, Konarska MM (2000). Specific HDV RNA-templated transcription by pol II in vitro. RNA, 6: 41-54.

Gerin JL, Casey JL, Purcell RH (2001). Hepatitis Delta Virus. In: Howley PM, (ed) Fields' Virology. Lippincott Williams & Wilkins, Philadelphia, pp 3037-3050.

Glenn JS, Watson JA, Havel CM, White JO (1992). Identification of a prenylation site in the delta virus large antigen. Science. 256: 1331-1333.

Greco-Stewart VS, Thibault CS, Pelchat M (2006). Binding of the polypyrimidine tract-binding protein-associated splicing factor (PSF) to the hepatitis delta virus RNA. Virology. 356: 35-44.

Gudima S, Wu SY, Chiang CM, Moraleda G, Taylor J (2000). Origin of hepatitis delta virus mRNA. J. Virol. 74: 7204-7210.

He LF, Ford E, Purcell RH, London WT, Phillips J, Gerin JL (1989). THe size of the hepatitis delta agent. J. Med. Virol. 27: 31-33.

Hsieh SY, Taylor J (1991). Regulation of polyadenylation of of hepatitis delta virus antigenomic RNA. J. Virol. 65: 6438-6446.

Hwang SB, Lee CZ, Lai MM (1992). Hepatitis delta agent expressed in recombinant baculoviruses: comparison of biochemical properties and post-translational modifications between the large and small formas. Virology. 190(1): 413-422.

Jeng KS, Su PY, Lai MM (1996). Hepatitis delta antigen enhances the ribozyme activities of hepatitis delta virus RNA in vivo. J. Virol. 70: 4205-4209.

Lazinski DW, Taylor JM (1993). Relating structure to function in the hepatitis delta virus antigen. J. Virol. 67: 2672-2680.

Lee CZ, Chen PJ, Lai MMC, Chen DS (1994). Isoprenylation of large hepatitis delta antigen is necessary but not sufficient for hepatitis delta assembly. Virology. 199: 169-175.

Li YJ, Stallcup MR, Lai MM (2004). Hepatitis delta virus antigen is methylated at arginine residues, and methylation regulates subcellular localization and RNA replication. J. Virol. 78: 13325-13334.

Macnaughton TB, Shi ST, Modahl LE, Lai MM (2002). Rolling circle replication of hepatitis delta virus RNA is carried out by two different cellular RNA polymearses. J. Virol. 76: 3920-3927.

Modahl LE, Macnaughton TB, Zhu N, Johnson DL, Lai MMC (2000). RNA-dependent replication and transcription of hepatitis delta virus RNA involve distinct cellular RNA polymerases. Mol. Cell. Biol. 20(16): 6030-6039.

Moraleda G, Taylor J (2001). Host RNA polymearse requirements for transcription of the human hepatitis delta virus genome. J. Virol. 75: 10161-10169.

Mu JJ, Wu HL, Chiang BL, Chang RP, Chen DS, Chen PJ (1999). Characterization of the phosphorylated forms and the phosphorylated residues of hepatitis delta virus delta antigens. J. Virol. 73: 10540-10545.

Mu JJ, Tsay YG, Juan LJ, Fu TF, Huang WH, Chen DS, Chen PJ (2004). The small delta antigen of hepatitis delta virus is an acetylated protein and acetylation of lysine 72 may influence its cellular localization and viral RNA synthesis. Virology. 319: 60-70.

Nie X, Chang J, Taylor J (2004). Alternative processing of hepatitis delta virus antigenomic RNA transcripts. J. Virol. 78: 4517-4524.

Perrotta AT, Been MD (1991). A pseudoknot-like structure required for efficient self-cleavage of hepatitis delta virus RNA. Nature. 350: 434-436.

Peter M, Nakagawa J, Doree M, Labbe JC, Nigg EA (1990). Identification of major nucleolar proteins as candidate mitotic substrates of cdc2 kinase. Cell, 60(5): 791-801.

Rosenstein SP, Been MD (1991). Evidence that genomica and antigenomic RNA self-cleaving elements from hepatitis delta virus have similar secondary structures. Nucl. Acids Res. 19: 5409-5416.

Ryu WS, Netter HJ, Bayer M, Taylor J (1993). Ribonucleoprotein complexes of hepatitis delta virus. J. Virol. 67: 3281-3287.

Sharmeen L, Kuo MY, Taylor J (1989). Self-ligating RNA sequences on the antigenome of the human hepatitis delta virus. J. Virol. 63: 1428-1430.

Sheu GT (2002). Initiation of hepatitis delta virus (HDV) replication: HDV RNA encoding the large delta antigen cannot replicate. J. Gen. Virol. 83(10): 2507-2513.

Sureau C, Guerra B, Lanford RE (1993). Role of the large hepatitis B virus envelope protein in infectivity of the hepatitis delta virion. J. Virol. 67: 366-372.

Szebeni A, Herrera JE, Olson MO (1995). Interaction of nucleolar protein B23with peptides related to nuclear localization signals. Biochemistry. 34(25): 8037-8042.

Tan KP, Shih KN, Lo SJ (2004). Ser-123 of the large antigen of hepatitis delta virus modulates its cellular localization to the nucleolus, SC-35 speckles or the cytoplasm. J. Gen. Virol. 85: 1685-1694.

Taylor JM (1996). Hepatitis Delta virus and its replication. In: Fields BN, Knipe DM, and Howley PM, eds. Fields Virology, 3rd ed. Philadelphia, Lippincott - Raven, 2809-2818.

Tavanez J, Cunha C, Silva MC, David E, Monjardino J, Carmo-Fonseca M (2002). Hepatitis delta virus ribonucleoproteins shuttle between the nucleus and the cytoplasm. RNA, 8(5): 637-646.

Tiollais P, Pourcel C, Dejean A (1985). The hepatitis B virus. Nature, 317: 489-495.

Yamaguchi Y, Filipovska J, Yano K, Furuya A, Inukai N, Narita T, Wada T, Sugimoto S, Konarska MM, Handa H (2001). Stimulation of RNA polymerase II elongation by hepatitis delta antigen. Science, 293: 124-127.

Yamaguchi Y, Delehouzee S, Handa H (2002). HIV and hepatitis delta virus evolution takes different paths to relieve blocks in transcriptional elongation. Microbes Infect. 4(11): 1169-1175.

Yeh TS, Lo SJ, Chen PJ, Lee YH (1996). Casein kinase II and protein kinase C modulate hepatitis delta virus RNA replication but not empty viral particle assembly. J. Virol. 70: 6190-6198.