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Hepatitis delta virus

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Abstract

Hepatitis delta virus (HDV) is a sub-viral agent that is dependent for its life cycle on hepatitis B virus (HBV). The help it obtains from HBV is limited to the sharing of envelope proteins. These proteins are needed to facilitate the assembly of the HDV genome into new virus particles, and in turn, to allow the attachment and entry of HDV into new host cells. In other respects, the replication of the small single-stranded circular RNA genome of HDV is independent of HBV. HDV genome replication produces two forms of a RNA-binding protein known as the long and small delta antigens (Ag). All other proteins needed for HDV genome replication, especially the RNA-directed RNA polymerase activity, are provided by the host cell. This mini-review article is a mixture of personal perspective and speculations about the future of HDV research. It starts with a brief overview of HDV and its replication, notes some of the major unresolved questions, and directs the interested reader to more detailed reviews.

Contents

History, epidemiology, prevention and treatment

HDV was discovered in 1977 among a group of Italian patients chronically infected with HBV, who developed serious episodes of acute liver disease (Rizzetto et al., 1977). By 1980, it was shown that HDV was an infectious agent responsible for exacerbation of liver disease in these patients (Rizzetto et al., 1980a). Both HBV and HDV are blood-borne infections. If a naive individual receives both viruses, it is referred to as a co-infection. Alternatively, HDV infection of an individual already chronically infected with HBV is referred to as a super-infection (Farci, 2003). Worldwide, above 460 million people are chronically infected with HBV and of these, it is speculated that around 20 million also have chronic HDV (Radjef et al., 2004). Acute HDV super-infections have a much greater risk of evolving into a fulminating hepatitis with liver failure than HBV alone. Chronic HDV infections are associated with more rapidly progressing liver damage than patients infected with HBV alone (Farci, 2003).

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Epidemiologic studies of HDV show that it is present in many different countries. Nucleotide sequence analyses of such isolates has allowed sub-classification into what was initially just three genotypes and more recently into a total of seven genotypes (Radjef et al., 2004).

Vaccination against HBV will also protect against HDV. Treatment of chronic HDV infections is currently limited to extensive alpha-interferon therapy or in extreme situations, to liver transplantation (Farci, 2003). Treatment with antivirals, such as lamivudine, that are known to reduce HBV titers, have not been successful in reducing HDV titers (Hoofnagle, 1998).

In mouse model systems (as also discussed later), isoprenylation inhibitors have been shown to transiently reduce assembly of HDV (Bordier et al., 2002) but it is not yet foreseeable that this kind of strategy will be used in patients because of potential toxicity (Taylor, 2003). Ribavirin, which in combination with interferon is the major antiviral therapy for chronic hepatitis C virus infections, has not been effective for treatment of HDV infections. This is puzzling since ribavirin will block HDV genome replication in cell culture (Choi et al., 1989; Rasshofer et al., 1991).

Experimental models and pathogenicity

HDV infection and replication can be achieved experimentally in the chimpanzee using HBV as the helper virus (Rizzetto et al., 1980b). Also, if the helper virus is replaced by woodchuck hepatitis B virus (WHV), replication can also be achieved in the Eastern woodchuck (Ponzetto et al., 1984). In mice that have been transplanted with human hepatocytes, HDV replication can also be achieved (Ohashi et al., 2000). If mice transgenic for HBV sequences are injected with HDV, HDV genome replication and assembly of new virus particles will take place, but virus spread does not occur (Bordier et al., 2003). Finally, if normal mice are injected with HDV, a limited amount of infection in the liver takes place, but again, there is no viral spread (Netter et al., 1993).

Since the genome of HDV can replicate in animal cells independent of HBV, it is not surprising that many such experimental systems are possible. Apparently without exception, transfection of mammalian cells with HDV cDNA will lead to RNA-directed genome replication. In contrast, transfection with HDV RNA, either natural genomic RNA or in vitro transcribed genomic or antigenomic RNAs, will not initiate replication. However, this blockage can be overcome in several different ways: If the HDV RNA is co-transfected with δAg (Dingle et al., 1998), or mRNA for δAg (Modahl and Lai, 1998) or if the recipient cell is already expressing δAg (Glenn et al., 1990), HDV genome replication will take place.

In all of the above experimental systems, HDV genome replication gradually declines due to the appearance with time of altered forms of δAg that either do not support replication or worse, act as dominant negative inhibitors of replication. The most obvious example of this, as discussed in a later section, is the larger form of δAg that arises because of a specific RNA-editing event. Other forms of δAg can also arise due to inappropriate RNA-editing and also via mis-incorporation during RNA-directed RNA synthesis. To obviate such self-limiting replication, a recent study described a new experimental system (Chang et al., 2005a). A cell line was made to conditionally express (under tetracycline control) a genetically stable form of the essential small δAg. These cells were transfected with an HDV cRNA, one that had been altered so that δAg species did not occur. Thus, in the uninduced state, low levels of HDV genome replication continued for at least 2 years, due to leaky expression of δAg mRNA for the uninduced promoter, and without any deleterious effects on the host cell. Furthermore, when higher levels of δAg were induced in the cells, high levels of HDV RNAs were transcribed and accumulated. Unexpectedly, this led to a cell-cycle arrest in G1/G0 within 2 days, and to detachment and cell death within 6 days. Thus, RNA-editing with the appearance of large δAg and down regulation of replication seems necessary for cell survival.

In this system, expression of the δAg alone did not have a cytopathic effect. This absence of a cytopathic effect is just as others have reported for mice made transgenic for δAg-S or δAg-L (Guilhot et al., 1994). In contrast, the situation where HDV genomic replication is induced and cells die, may be a model for replication-associated cytopathogenicity. It might help explain how in vivo, at the peak of natural and experimental acute infections, HDV replication can be so pathogenic (Farci, 2003; Gowans and Bonino, 1993). In chronic infection, virus production and pathogenicity are much lower. Thus, genome replication in vivo is initially fast enough that cells die before adequate levels of large δAg are produced to suppress HDV RNA synthesis.

Virus structure and assembly

As mentioned earlier, HDV makes use of the three HBV envelope proteins. These have a common *carboxyl*-terminus, and they are referred to by their sizes as large, middle and small, or L, M and S. Each protein exists with and without a site-specific N-linked carbohydrate modification. HBV particle assembly, even in the absence of HDV, is very inefficient in that most of the assembled particles are empty and do not contain the HBV nucleocapsid structure or genome. The serum of an infected patient may contain 1000 to 1,000,000 empty particles for every one that contains the viral nucleocapsid (Ganem, 1991). Most of the empty particles are spheres and filaments, about 22 nm in diameter, that are composed predominantly of the S protein. Only when the L protein is also present can the nucleocapsid be enveloped to produce infectious 40 nm particles. Furthermore, the unique amino-terminus of the L protein is needed for these particles to be infectious. The M protein is a minor component and is apparently not necessary either for assembly or infectivity.

HDV assembly makes use of the excess production of envelope proteins, and inserts the HDV genomic RNAs and about 70 molecules of δAg into particles containing L, M and S. The S protein is sufficient for particle assembly, but the particles so produced are not infectious. As with the infectious

HBV, the L protein is also needed for infectivity (Sureau et al., 1993).

It would seem that HBV and HDV use very similar mechanisms for virus attachment and entry. However, some caution is needed since in neither case do we know the host receptor(s). We also do not know the post-entry steps. An additional complication is that infectious HDV can be assembled from HBV proteins that are not carbohydrate modified, but this is not true for HBV (Sureau et al., 2003).

No cryo-electron microscopy has yet been reported for HBV or HDV particles.

RNAs

As represented in Fig. 1, The HDV RNA packaged into virions is by definition, the genome. It is a small 1679 nucleotide single-stranded RNA with a circular conformation. Both theoretically and experimentally, this RNA can fold on itself, using 74% intra-molecular base-pairing, to form an unbranched rod-like structure (Kuo et al., 1988; Wang et al., 1986).

HDV genome replication is via RNA-directed RNA synthesis. Unlike the helper virus, HBV, there are no DNA intermediates (Chen et al., 1986). During HDV genome replication, up to 300,000 copies of genomic RNA accumulate per cell (Chen et al., 1986). Also detected, in about 10-fold lower amounts is an exact complement of the genome, called the antigenome (Chen et al., 1986). As indicated in Fig. 1, the genome and antigenome each contain a single ribozyme domain of about 85 nucleotides (Ferre-D'Amare et al., 1998; Sharmeen et al., 1988). Crystal structures have now been obtained for the genomic ribozyme both in the pre- and post-cleaved states. Such findings show that a conformational switch occurs as a consequence of cleavage and also clarify the role of a divalent metal ion in the cleavage event (Ferre-D'Amare et al., 1998; Ke et al., 2004).

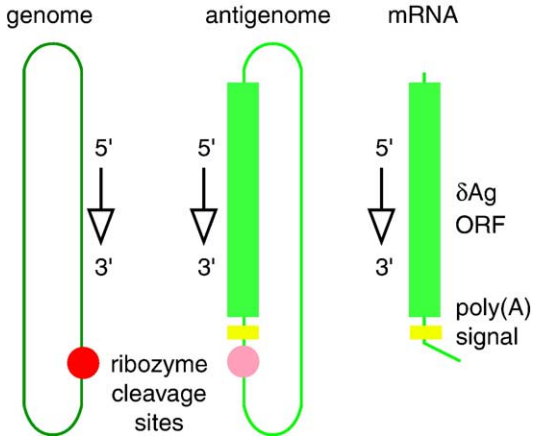

Fig. 1. The three HDV RNAs that accumulate during genome replication. The genome and its exact complement, the antigenome, are 1679 nucleotide long single-stranded RNA circles, each possessing a ribozyme, with cleavage sites indicated by red circles. The antigenome also contains the open reading frame for the δAg and a poly(A) signal, as indicated. However, the δAg is actually translated from a 800-nucleotide mRNA, that has a 5′-cap and a 3′-poly(A) tail.

A third HDV RNA, typically 500 times less abundant than the genome, is of the same polarity as the antigenome. As indicated in Fig. 1, this RNA is linear and only about 800 nucleotides in length. It has a 5′cap structure and a 3′-poly(A) tail consistent with its role as mRNA for the translation of the δAg (Nie et al., 2004).

As with the mRNA transcribed from a DNA template, the HDV mRNA arises via post-transcriptional processing. Near to the site of poly(A) addition on the antigenomic RNA is a poly(A) signal, AAUAAA, and other sequence features, just as seen for the processing of host mRNAs. Mutation of the HDV poly(A) signal interferes with the accumulation of the mRNA (Nie et al., 2004).

Fig. 2 is a representation of the mechanism of genome replication, with emphasis on RNA transcription and processing.

There are other RNAs produced during HDV replication. Less abundant than monomers of the genome and antigenome are dimers and trimers (Chen et al., 1986). Also, using pulse-labeling conditions, even higher order multimers can be detected (Macnaughton and Lai, 2002a). Some of these are considered to be largely processed down to linear monomers by the HDV ribozymes, and further processed to circles, possibly by a host RNA ligase activity (Reid and Lazinski, 2000).

Delta antigen(s)

Initiation of HDV genome replication absolutely requires the presence of the 195 amino acid δAg species (Chao et al., 1990). This protein has been ascribed several properties, some of which could contribute to it being essential (Taylor, 2006). In addition to its RNA-binding ability, which may or may not be specific for the HDV rod-like structure (Chao et al., 1991), there is also evidence that the protein can facilitate in vitro elongation of RNA transcription by host RNA polymerase II (Yamaguchi et al., 2001).

During HDV genome replication in the nucleus, some of the new HDV RNAs get post-transcriptionaly edited by an enzyme of the class known as adenosine deaminase acting on RNA, or ADAR. The small form of ADAR-1, is considered to be the specific enzyme that acts on HDV RNA (Wong and Lazinski, 2002). It changes adenosine to inosine. This change is immortalized by subsequent rounds of RNA-directed RNA synthesis, as adenosine to guanosine.

The site of one such editing change corresponds to an adenosine in the middle of the UAG amber termination codon for the small δAg. The new codon, UGG, is translated as tryptophan, to produce a 19-amino acid longer form of δAg. Unlike the small form, this large form does not support genome replication and in fact, under certain conditions can be a dominant negative inhibitor (Chao et al., 1990), although there is some contrary opinion (Macnaughton and Lai, 2002b; O'Malley and Lazinski, 2005). However, what is clear is that the large form has a separate and an essential role in the HDV life cycle. It is needed for the assembly of new particles using the HBV envelope proteins (Chang et al., 1991).

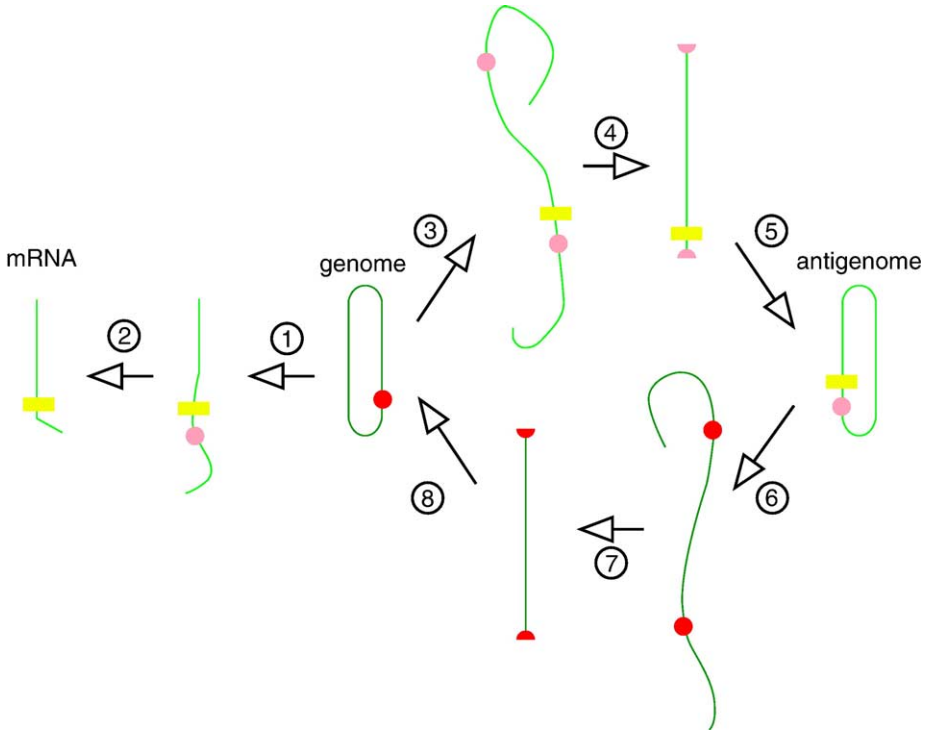

Fig. 2. A model of HDV genome transcription and processing. The three RNAs of HDV, and their associated sequence features, are as indicated in Fig. 1. (1) The genomic RNA acts as a template for the transcription of an antigenomic RNA. (2) This nascent RNA transcript has the same 5′-end as the mRNA (Gudima et al., 2000). It subsequently undergoes 5′-capping and 3′-poly(A) processing. (3) The genomic RNA acts as a template for other antigenomic RNA transcripts, some of which since they are transcribed from a circular RNA template can achieve greater than unit-length. (4) These are processed in an alternative fashion to step 2. They undergo ribozyme cleavage to release unit-length linear antigenomes. (5) These in turn, fold into the rod-like structure and are ligated to produce new antigenomic RNA circles. (6–8) As in steps 4–6, the new antigenomic RNA can act as template for the transcription and processing of new genomic RNAs.

Even more surprising is that in the extra 19 amino acids of the large δAg, there is a unique cysteine located 4 amino acids from the *carboxyl*-terminus. It acts as a site for isoprenylation (Glenn et al., 1992) and this modification is essential for the ability of the large δAg to support HDV assembly (Bordier et al., 2003).

Various other post-translational modifications of the large and small delta proteins have also been described. There is evidence for phosphorylation (Chang et al., 1988; Mu et al., 1999), methylation (Li et al., 2004) and acteylation (Mu et al., 2004). Some of the modification sites have been mapped and certain of the modifications may be relevant to the HDV life cycle.

RNA polymerase requirements

Since HDV encodes no more than the delta protein(s), genome replication requires host polymerase activity. Much data support the interpretation that host RNA polymerase II, which is normally a DNA-directed RNA polymerase, is redirected to carry out HDV RNA-directed transcription.

This picture is not, however, without some complications, as can be seen from a recent review by Lai (2005). It is agreed that Pol II is involved in the transcription to produce both genomic RNA and mRNA. However, some data have been interpreted as evidence that a polymerase resistant to >100 micrograms/ml of amanitin, thus like Pol I, is involved in transcription to produce antigenomic RNA. This has led to a very complex model of rolling-circle replication, with genomic RNA sometimes acting as a template for pol II and mRNA and other times acting as a template for another enzyme, to make antigenomic RNA.

Template-switching and recombination

It is most likely that during normal HDV genome replication, the RNA templates used most frequently are unit-length circular RNAs. However, some of the intracellular HDV RNAs, especially the precursors to circular monomers, are likely to be linear RNAs. And, from experimental studies, there is evidence that replication can be initiated from HDV RNA templates that are linear (Gudima et al., 2004). This process involves intra-molecular template-switching during transcription, and sometimes is associated with small deletions of HDV sequences and/or insertions of non-templated nucleotides (Chang and Taylor, 2002). In another study, pairs of HDV RNAs, each much less than unit-length but together representing at least one copy of the entire HDV sequence, were transfected into cells and full-length replicating genomes were reconstituted, presumably by inter-molecular template-switching (Gudima et al., 2005).

Given the data for template-switching by a host RNA polymerase on an RNA template, it might also be expected that inter-molecular recombination could occur during natural

infections. This has been reported to occur in patients infected with two different genotypes of HDV (Wu et al., 1999) and also in cells transfected with two RNAs of different genotype (Wang and Chao, 2005).

Virus attachment and entry

Since HDV shares the envelope proteins of HBV, many labs think that the two viruses would use a very similar, if not identical, attachment and entry mechanism. There is evidence that a unique region near the amino-terminus of the HBV L protein is needed for entry by both viruses (Barrera et al., 2005). Puzzlingly, when the N-linked carbohydrate modification of L, M and S is removed, the assembled HBV is non-infectious while HDV remains infectious (Sureau et al., 2003). More studies are needed on the attachment and entry mechanisms for both viruses. Certainly, a better understanding of virus entry might lead to additional options for anti-viral therapy, for example, as used in anti-HIV therapies (Kilby et al., 1998).

Also missing are data on post-entry events. Does HDV (or HBV) entry require an endocytic pathway? Is there a need for acidification within an endosomal compartment? And, once released, how does the viral ribonucleoprotein make its way to the nucleus for transcription; is this transport mediated by microtubules?

Viroid analogy

Among the small subviral agents that can infect plants is a group of small single stranded non-coding RNAs that are known as viroids (Flores et al., 2004; Tabler and Tsagris, 2004). As reviewed elsewhere, there are many similarities between these viroids and HDV (Taylor, 1999). Such similarities have and will continue to lead to interesting new findings. For example, the search for the HDV ribozymes was motivated by certain earlier viroid findings. Also, new studies of HDV genome replication, as described earlier in the manuscript, have to some extent made the replication of HDV in cultured cells much more like that of a plant viroid (Chang et al., 2005b).

Nevertheless, there are still major differences between the viroids and HDV: (i) The viroids have much smaller genomes (typically 200–450 nucleotides). (ii) The viroids do not encode any protein. (iii) The viroids do not have any known helper virus.

More data are needed for both the viroids and HDV to explain what is unique about RNAs that are recognized and copied by host RNA polymerase(s).

Conclusions and outlook

As a cause of infectious disease HDV is coming under control, largely due to the preventative vaccination against HBV. Also, the majority of cases of chronic HDV is largely holdovers from pre-vaccination times, and as such, is progressively decreasing in number.

As a source of intriguing molecular biology and molecular virology, HDV continues to be very special. The best examples of this are its ribozymes, the essential ADAR-editing, and of course, the RNA-directed RNA synthesis by a redirected host RNA polymerase(s). The latter process is the one that needs significant clarification and less controversy. We and others have sought for an in vitro Pol II transcription system using HDV RNAs. To date, all that has been reproducibly achieved is modest 3′-end addition reactions (Beard et al., 1996; Filipovska and Konarska, 2000; Gudima et al., 2000; Yamaguchi et al., 2001).

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