CHAPTER 3

Structure and Replication of Hepatitis Delta Virus RNA

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Abstract

This review focuses on the RNAs of HDV, with emphasis on RNA structure, RNA transcription, and post-transcriptional RNA processing. Included is an evaluation of two current models of HDV RNA replication.

Introduction and Scope

Hepatitis delta virus (HDV) was first discovered in 1977 through the work of Rizzetto and coworkers.[1] Around 1986 several other labs began to work on the molecular virology of this agent and over and over, HDV has provided us with intriguing and unique phenomena in molecular virology. There are still important questions that need to be resolved. However, as a problem of natural infections in humans, HDV is apparently slowly "vanishing".[2]

The molecular biology of the HDV RNAs and their mechanism of replication depend upon the production of two related virus-encoded proteins, the small and large forms of the delta antigen (δAg), referred to here as δAg-S and δAg-L, respectively. The properties of these essential proteins are the focus of Chapter 4. In the present chapter, the focus will be on the RNAs of HDV, with consideration of such features as structure, transcription, post-transcriptional processing, and stabilization. The reader might also want to consider earlier reviews of these topics.[3-10]

The RNAs

Many different complete HDV RNA sequences have now been reported.[11-13] Most sequences are at or about 1,679 nucleotide (nt) in length. We will in this review use the numbering system of Kuo et al.[13] The origin of this numbering is indicated in Figure 1. For the genomic RNA the numbering increases for the 5'-3' direction. For the antigenome, the numbering decreases for the 5'-3' direction.

Consider now the three HDV RNA species that get the most attention. As diagrammed in Figure 1, they are the genome, the antigenome, and the mRNA. The RNA species that is assembled into virus particles is, by definition, the genome. It is a single-stranded RNA with a circular conformation. Within cells where this genome is replicating there is also present typically 5-20 fold lower amounts of the antigenome, an exact complement of the genome. The third

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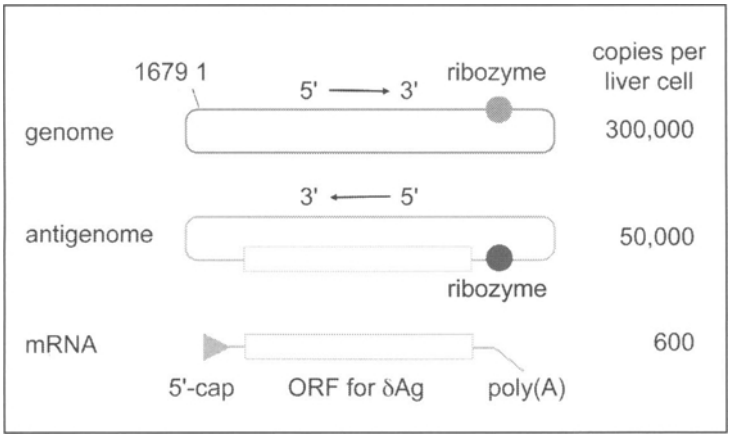

Figure 1. Three processed RNAs of HDV. These are the genome, antigenome and mRNA. Indicated on the genome and antigenome are the positions of ribozyme cleavage. Indicated on the mRNA, with its open reading frame for δAg, are the 5'-cap and 3'-poly(A). The abundances of each species are as reported previously.[16] Indicated is the position of the origin of the 1,679 nucleotide sequence of Kuo et al.[13]

RNA species is of the same polarity as the antigenome. It is linear, 5'-capped[14] and 3'-polyadenylated.[15] Its length of about 800 nt spans the open reading frame for δAg and is considered to be its mRNA. As will be explained in more detail, not just the mRNA but all three of these RNAs have undergone one or more forms of post-transcriptional RNA processing.

Actually, during HDV RNA replication there are minor amounts of yet other processed RNAs. These include relatively low amounts of dimers and even trimers of the unit-length, for both genomic and antigenomic polarity.[16] For these multimers as well as for the monomers, the majority of the RNA is in a circular conformation.

The 5'-end of the mRNA was initially mapped at position 1631 using primer extension assays.[15] In later studies using 5'-RACE procedures, it was mapped to position 1630.[17] This is the predominant 5'-end but there seem to be other sites that are less abundant and less specific.[17] Uncertainty arises because in many of the early studies the mRNA was of low abundance, typically <2% relative to the antigenomic RNA. At the right side of Figure 1 is indicated what has been deduced for the number of the three HDV RNAs per average hepatocyte in an HDV infected liver.[16]

While the unit-length genomic and antigenomic RNAs are primarily in a circular conformation, there are unit-length linear forms and their nature may be complex. Some may be species whose ends have been defined by ribozyme cleavage and have yet to be circularized. Others may be circles that have been reopened by ribozyme cleavage or exonuclease action. In one study, many 5'-ends of linear genomic monomers detected within the liver of an infected animal were mapped to a specific site that was not a site of ribozyme cleavage.[18] As considered later, the site is more likely to be a site of endonucleolytic opening on preformed circles than a site of initiation of transcription.

Yet another class of HDV RNAs must exist. These are the unprocessed and partially processed linear RNAs of both genomic and antigenomic polarity. Some studies have detected species of much greater than unit-length that may be examples of unprocessed nascent transcripts.[19] Presumably because of their transitory nature and/or the techniques used, the unprocessed species are more difficult to detect and characterize. Such RNAs should include species containing the anticipated 5'-ends of nascent transcripts. However, at this time we have no data for the detection of 5'-ends for genomic RNAs. As explained below, the 5'-end of the mRNA species might correspond to an initiation site of antigenomic RNA.