CHAPTER 4

Hepatitis Delta Antigen:

Biochemical Properties and Functional Roles in HDV Replication

Michael M.C. Lai*

Hepatitis delta antigen (HDAg) was first detected in the nucleus of the hepatocytes of some patients infected with hepatitis B virus (HBV).[68] The presence of HDAg was frequently associated with severe hepatitis. This antigen was initially thought to be a previously unrecognized HBV-encoded antigen, but later was found to be associated with a novel virus, hepatitis delta virus (HDV).[69] HDAg is an internal component of the HDV virion particles, and, together with the viral RNA genome, forms viral nucleocapsid.[2] There are approximately 70 HDAg molecules per RNA molecule in each virion particle,[72] but the precise structure of the nucleocapsid has not been determined. HDAg in virus preparations from most of the patients usually consists of two distinct forms of different size (27 and 24 kDa, termed the large and the small HDAg, L- and S-HDAg, respectively). The nucleocapsid is released from the virus particle after the latter is treated with nonionic detergents.[72] Besides this structural role, HDAg also plays a very critical role in the HDV life cycle by participating in various steps of viral replication, including viral RNA synthesis (by S-HDAg), virus assembly (by L-HDAg) and others. HDAg is the only known functional protein encoded by HDV RNA. It is encoded by the antigenomic-sense strand of HDV RNA, but is translated from a 0.8-kb mRNA, which is transcribed from the viral genomic RNA.

Structural and Functional Domains of HDAg

The S-HDAg (195 amino acids) and L-HDAg (214 amino acids) are identical except that L-HDAg has 19 additional amino acids at the C-terminus. The N-terminal two-thirds of the protein are highly basic, while the C-terminal one-third is relatively uncharged.[9] At least four structural and functional domains have been identified in HDAg (Fig. 1).

RNA-Binding Domains

There are several RNA-binding domains within HDAg. The first identified one is mapped in the middle one-third of the protein.[46] This domain consists of two stretches of arginine-rich motif (ARM),[45] which had been identified in several other viral RNA-binding proteins, such as *rev* and *tat* of human immunodeficiency virus.[38] Both of the ARM sequences and a spacer

*Michael M.C. Lai—Department of Molecular Microbiology and Immunology, University of Southern California, Keck School of Medicine, Los Angeles, California 90033, U.S.A.; and Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan 115. Email: michlai@usc.edu

Hepatitis Delta Virus, edited by Hiroshi Handa and Yuki Yamaguchi.

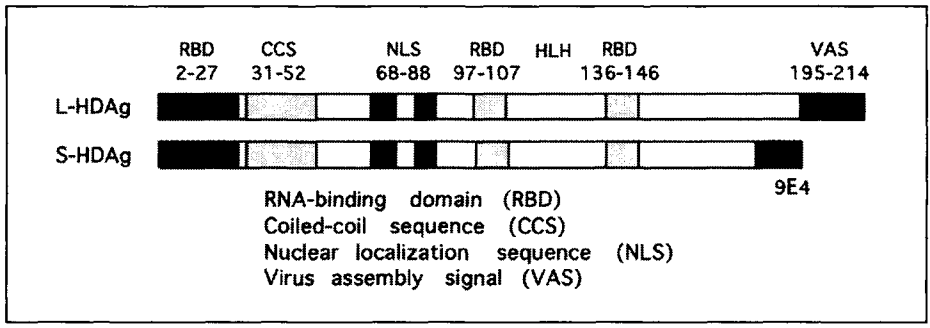

Figure 1. Schematic diagram of the functional domains of the large and small hepatitis delta antigen.

sequence of appropriate length, which contains a helix-loop-helix (HLH) motif,[10] are required for RNA binding.[45] Another potential RNA-binding motif is found in the N-terminal region of the protein (aa 2-27).[65] The RNA-binding activity is the basis of the various biological functions associated with HDAg, such as RNA replication and RNA transport. Studies on the HDAg-mediated HDV RNA transport indicated that any one of these three RNA-binding motifs is sufficient for mediating HDV RNA-HDAg binding,[19] although the requirement for RNA binding in vitro appears to be more stringent.

HDAg is a general RNA-binding protein,[12,34,46] with a particularly high binding affinity for HDV RNA; however, the binding specificity is not absolute. HDAg binds to both genomic and antigenomic strands equally efficiently. In the virion particles, HDAg is bound to HDV RNA,[46] which is exclusively of genomic strand. Thus, there are other factors which contribute to the specificity of RNA packaging into the virus particles. The HDAg-RNA interaction plays a role not only in the structural organization of virion particles, but also in HDV RNA replication (see below).

Nuclear Localization Signal (NLS)

The main NLS of HDAg is located in the N-terminal one-third (aa 68-88) of the protein.[88] This domain contains two stretches of basic amino acids, both of which are required for targeting HDAg to the nuclei. HDAg carries the incoming HDV RNA to the nucleus, where RNA replication occurs.[19] HDAg utilizes the classical nuclear-importing machinery of karyopherin $\alpha 2\beta$ pathway.[19] It has been shown that the HDV RNA-HDAg complex can shuttle in and out of the nucleus,[77] suggesting that HDAg may also have a nucleus-exporting function. However, so far, the nucleus-import and -export domains within HDAg can not be separated. Significantly, a nucleus-exporting signal has been identified in the C-terminal 19 amino acids of L-HDAg, which allows L-HDAg to be located in the cytoplasm to interact with HBsAg.[42] This domain by itself can serve as a nuclear export signal in Xenopus oocytes. This property is consistent with the functional role of L-HDAg in virus assembly, which likely takes place in the cytoplasm. Obviously, there must be a second nucleus-exporting signal which is present in both S- and L-HDAg to account for the export of HDV RNA (particularly the genomic strand) immediately after synthesis.[54] Since HDV RNA likely does not have an intrinsic nuclear localization signal, the final localization of HDV RNA is likely determined by the properties of HDAg, which complexes with HDV RNA. The nucleus-export of L-HDAg and HDV RNA utilizes a Crm1 (chromosome region maintenance 1)-independent pathway.[42] Deletion of the Arg-rich motif also leads to the accumulation of HDV RNA in the cytoplasm, indicating that the nuclear localization of HDV RNA requires the interaction between HDV RNA and HDAg.[19]