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Functional roles of hepatitis delta antigen in virus packaging and RNA replication in hepatitis delta virus

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Hepatitis delta virus (HDV) is a hepatotropic virus that causes severe or fulminant hepatitis. The virus is defective and always associated with hepatitis B virus (HBV) infections since HDV requires HBV to provide hepatitis B surface antigen (HBsAg) to form the 36-nm HDV particle. HDV particle consists of an internal hepatitis delta antigen (HDAg) and a circular RNA of 1.7 kb, which together form a nucleocapsid structure. The genomic RNA forms a rod-like structure and shares many structural features and some sequence similarity with viroid or virusoid RNAs. HDV RNA replicates by RNA-dependent RNA synthesis via a double rolling circle mechanism and has autocatalytic cleavage and ligation activities. HDAg is the only protein encoded by HDV from its antigenomic RNA. It consists of two protein species of 24 kDa (small HDAg, SHDAg) and 27 kDa (large HDAg, LHDAg). The two proteins are identical in sequence, except that LHDAg contains an additional 19 amino acids at its C-terminus. We have expressed both forms of the HDAg and demonstrated that both HDAgs are phosphoptotein localized exclusively in the nuclei. We have found that both protein species have equal RNA binding activities. It was also demonstrated that only the LHDAg was isoprenylated, while the small one was not.

The two protein species have distinctly different functions: The SHDAg is required for HDV RNA replication, while the LHDAg inhibits HDV RNA replication and initiates HDV virus assembly. To understand the molecular mechanism of HDV virion morphogenesis, we investigated the possible direct protein-protein interaction between HDAg and HBsAg. We demonstrated that HBsAg interacted specifically with the LHDAg but not with the SHDAg and this interaction required isoprenylates on the cysteine residue of the C terminus of the LHDAg. We also examined the effects of prenylation on the conformation and function of HDAg. We have found that the presence of prenylates masks a conformational epitope which is present in SHDAg but hidden in wild type LHDAg. We also have shown that conformational differences between the large and small HDAgs account for the differences in biological activities.

To further characterize the functional role of SHDAg in viral RNA replication, we transfected stable cell line expressing SHDAg with *in vitro* transcribed HDV RNA. We have found that the transfected RNA replicated very efficiently in the cell line stably expressing SHDAg, but not in the parental cell line without SHDAg. When HDV RNA was co-transfected with a SHDAg-encoding plasmid, no HDV RNA replication occurred, suggesting that HDAg had to be expressed early in the replication cycle in order for HDV RNA to replicate. The requirement for a stably expressing SHDAg prior to RNA transfection is unique to HDV RNA replication from a transfected RNA template.