INTERACTION BETWEEN HEPATITIC C VIRUS RNA-DEPENDENT RNA POLYMERASE AND NS5A PROTEIN: MECHANISM AND REGULATION

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Hepatitis C virus (HCV) RNA-dependent RNA polymerase (RdRp) can be inhibited by nonstructural protein 5A but the mechanism of the process was unknown. In the present work it was studied with the use of recombinant proteins obtained in sufficient quantities from tailor-made prokaryotic expression system. Here we show that interaction of NS5A protein with RdRp has no effect on RNA elongation but blocks initiation of RNA synthesis. The inhibition of RdRp by NS5A can be abolished by phosphorylation of the latter which occurs in infected cells. The study of *in vitro* phosphorylation of NS5A suggest that in eukaryotic cells NS5A is phosphorylated by casein kinases (CK) I and II, and by at least one unidentified protein kinase. Both forms of NS5A, obtained by phosphorylation with eukaryotic cell lysate and recombinant CKII, are unable to inhibit RdRp. CKII modifies four amino acid residues in NS5A C-terminal region, and Ser408 in particular. However, it does not alter NS5A RNA-binding activity or affinity to RdRp. Therefore, the effect of phosphorylation of NS5A on RdRp is likely to be determined by conformation of protein C-terminus. According to various methods, unphosphorylated full-length NS5A prevents binding of RdRp to RNA template whereas phosphorylation or truncation of C-terminal region restores initiation of active polymerase-template complexes formation. Hence, the NS5A phosphorylation may present a way of regulation of HCV replication. The significance of CKII for virus replication was conferred by RNA interference and by specific kinase inhibitor in HCV replicon system.