Hepatitis C Virus NS5A binds RNA-Dependent RNA polymerase NS5B and modulates RdRP activity

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HCV NS5B is RNA-dependent RNA polymerase (RdRP), the essential catalytic enzyme for HCV replication. Recently, NS5A has been reported to be important for the establishment of HCV replication *in vivo* by the adaptive mutations although its role in viral replication remains uncertain. Here, we report that purified bacterial recombinant NS5A and NS5B directly interact each other *in vitro* detected by glutathione-S-transferase (GST) pull-down assay. Furthermore, complex formation of these proteins transiently coexpressed in mammalian cells was detected by coprecipitation. Using terminally and internally truncated NS5A, two discontinuous regions of NS5A (aa 105-162 and aa 277-334) outside of the adaptive mutations were identified to be independently essential for the binding both *in vivo* and *in vitro*. We examined effect of His-NS5A on RdRP activity of the soluble recombinant NS5Bt *in vitro* (J. Biol. Chem., 273 : 15476, 1998). Wild NS5A weakly stimulated at first (when less than 0.1 molar ratio to NS5B), then inhibited the NS5Bt RdRP activity in a dose dependent manner. The internal-deletion mutants defective in NS5B-binding exhibited no inhibitory effect, indicating that the NS5B-binding was necessary for the inhibition. Taken together, our results support the idea that NS5A modulates HCV replication as a component of replication complex.

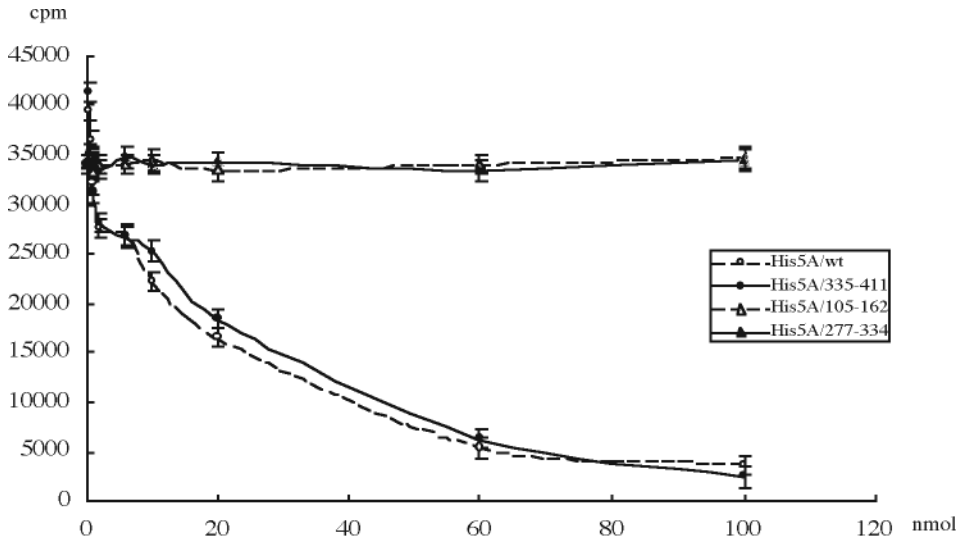

Figure NS5A modulates RdRP activity of NS5B

Ten nmol of purified GST-NS5Bt in the presence of 0, 0.2, 0.6, 1, 2, 6, 10, 20, 60, and 100 nmol of wild or internal-deletion His-NS5A were examined for poly (A)-dependent UMP incorporation assay as described.
Effect of His-NS5A/d163-221 was similar to that of the wild His-NS5A. Weak stimulation effects of wild and mutant NS5A proteins were observed when the molar ratio of His-NS5A to GST-NS5Bt is less than 0.1.