Effect of Interaction between Hepatitis C Virus NS5A and NS5B on the Hepatitis C Virus Replicon[1].

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HCV NS5A is important for the establishment of replication by adaptive mutations or localization, although its role in viral replication remains unclear. It was previously reported that NS5A interacts with NS5B via two regions of NS5A in the isolate JK-1 and modulates the activity of NS5B RdRp (Y. Shirota et al., J. Biol. Chem., 277:11149–11155, 2002), but the biological significance of this interaction has not been determined. We examined the effect of this interaction on HCV RNA replication with an HCV replicon system derived from the isolate M1LE (H. Kishine et al., Biochem. Biophys. Res. Commun., 293:993–999, 2002). We constructed three internal deletion mutants, M1LE/5Adel-1 and M1LE/5Adel-2, each encoding NS5A which cannot bind NS5B, and M1LE/5Adel-3, encoding NS5A that can bind NS5B. After transfection into Huh-7 cells, M1LE/5Adel-3 was replication competent, but both M1LE/5Adel-1 and M1LE/5Adel-2 were not. By scanning with 20 alanine-substituted clustered mutants within both NS5B-binding regions, only 5 of the 20 mutants were replication competent. Subsequently, we established more efficient replicon system by introducing a point mutation, S232I, into NS5A using cured Huh-7 cells as recipient cells. In this system, only the same five mutants were replication competent. These results strongly suggest that the interaction between NS5A and NS5B is critical for HCV RNA replication in the HCV replicon system. This improved system was applied to examine whether the 5 residues of NS5B indispensable for RdRP activity *in vitro* are critical in HCV replication. An alanine-substitution mutant of one of the 5 residues was replication incompetent in the HCV replicon system[2].

Reference 1: Shimakami, T., Hijikata, M. Luo, H., Ma, Y., Kaneko, S., Shimotohno, K., Murakami, S. (2004) J. Virol., 78: 2738-2748.
Reference 2: Ma Y, Shimakami T, Luo H, Hayashi N, Murakami S. (2004) J Biol Chem, 279(24):25474-25482.