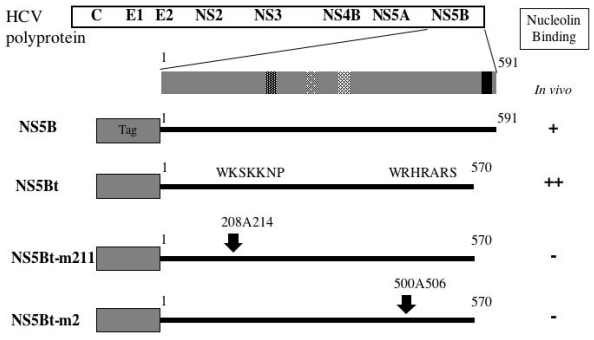
Direct Interaction between nucleolin and HCV NS5B[1].

M. Hirano, S. Kaneko, T. Yamashita, H. Luo, W. Qin, Y. Shirota, T. Nomura, K. Kobayashi, S. Murakami.

HCV NS5B is an RNA-dependent RNA polymerase (RdRP), a central catalytic enzyme in HCV replication. While studying the subcellular localization of a NS5B mutant lacking the C-terminal membrane-anchoring domain, NS5Bt, we found that expression of the GFP-fused form was exclusively nucleolar. Interestingly, the distribution of endogenous nucleolin changed greatly in the cells expressing GFP-NS5B, with nucleolin colocalized with GFP-NS5B in perinuclear regions in addition to the nucleolus. The interaction between nucleolin and NS5B was demonstrated by GST pull-down assay. The results indicated that C-terminal region of nucleolin was important for its binding to NS5B. Scanning a clustered- alanine substitution mutant library of NS5B revealed that two sequences of NS5B, aa 208-214 and aa 500-506, were both found to be indispensable for the nucleolin binding. We reported that the latter sequence is essential for oligomerization of NS5B which is a prerequisite for the RdRP activity. C-terminal nucleolin inhibited the NS5B RdRP activity in a dose dependent manner. Taken together, the binding ability of nucleolin may be involved in NS5B functions.

Reference 1: M. Hirano, S. Kaneko, T. Yamashita, H. Luo, W. Qin, Y. Shirota, T. Nomura, K. Kobayashi, S. Murakami. (2003) J. Biol. Chem., 278: 5109-5115.



Two sequences of NS5B are critical for nucleolin-binding.

The critical sequences of NS5B shown at the top of NS5Bt which were clustered alanine-substituted as shown at the bottom constructs[1].