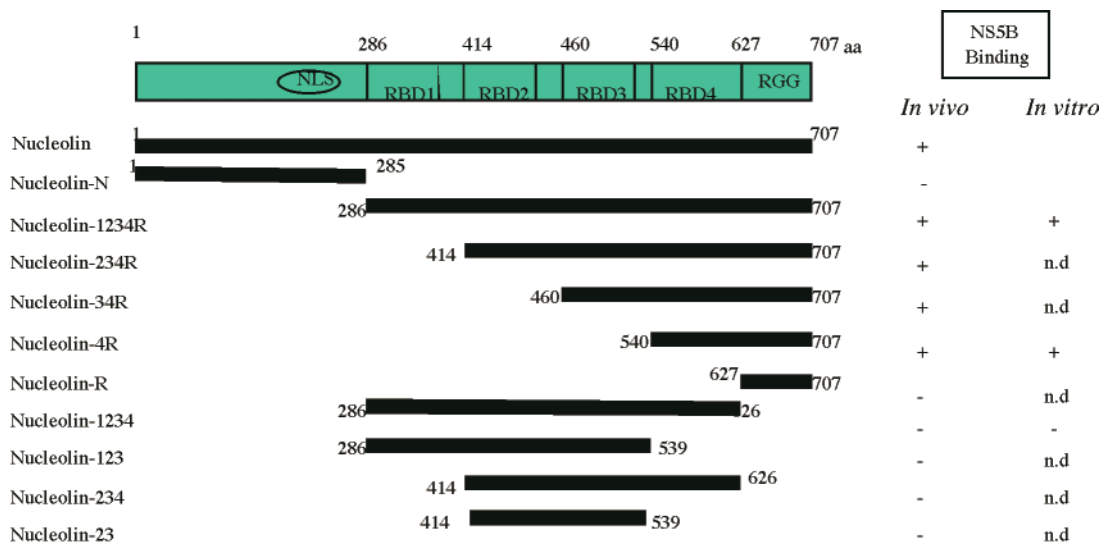


## Direct interaction between nucleolin and HCV NS5B

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Hepatitis C virus (HCV) is one of the major causative agents of hepatitis. Two envelope proteins Hepatitis C virus (HCV) NS5B is an RNA-dependent RNA polymerase (RdRP), a central catalytic enzyme in HCV replication. Previously, we identified the C-terminal 21 amino acid sequence as a membrane attachment domain. It is dispensable for RdRP activity but important for subcellular localization of NS5N. While studying the subcellular localization of a NS5B mutant lacking the C-terminal 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, suggesting that NS5B retained the ability to bind nucleolin.

The interaction between nucleolin and NS5B was demonstrated by GST pull-down assay. GST pull-down assay results indicated that C-terminal region of nucleolin was important for its binding to NS5B. Scanning clustered alanine substitution mutants library of NS5B revealed two sites on NS5B that bound nucleolin. 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 vitro* in a dose-dependent manner. The inhibition seemed to be dependent upon the ability to interact with NS5B since truncated forms of nucleolin that could not bind NS5B did not affect RdRP activity of NS5B. Taken together, the binding ability of nucleolin may be involved in NS5B functions.



**Figure** Structures of wild-type and deletion mutant forms of human nucleolin and characteristics of NS5B binding. Boxes represent the structure of different constructs of human nucleolin protein with the boundaries of amino acid residues indicated above. The activity of each nucleolin mutant to interact with NS5B is indicated by a plus or minus sign. NLS; nuclear localization signal. RBD; RNA binding domain.