

Oligomeric interaction of Hepatitis C Virus NS5B is critical for catalytic activity of RNA dependent RNA polymerase.

Weiping Qin, Hong Luo, Takahiro Nomura, Naoyuki Hayashi, Tatsuya Yamashita, and Seishi Murakami

HCV NS5B is an RNA-dependent RNA polymerase (RdRP), a central catalytic enzyme for HCV replication, which has the "palm and fingers" substructure. We recently identified 5 novel residues critical for RdRP activity (Hepatology, 33:728-737 (2001)). Among them, E18 and H502, far from the catalytic center, may be involved in conformational change(s) for RdRP activity as addressed in some "palm and fingers" enzymes. We examined the possibility that NS5B is oligomerized, and we could detect interaction between two different tagged NS5B proteins *in vitro* and transiently expressed in mammalian cells. By scanning 27 clustered and then point alanine substitutions *in vivo* and *in vitro*, E18 and H502 were found to be critical for the homomeric interaction *in vivo* and *in vitro*, strongly suggesting a close relationship between the oligomerization and RdRP activity of NS5B. All mutants with substitutions at these two residues failed to bind wild type NS5B, however E18H interacted with H502E *in vitro* and *in vivo*. Interestingly, the NS5B protein with either E18H or H502E did not exhibit RdRP activity, but a mixture of the two mutant proteins did. These results clearly indicate that these two residues of HCV NS5B are critical for the oligomerization that is prerequisite to RdRP activity.

Table Effects of NS5B mutants defective in oligomerization on the RdRP activity

Proteins	UMP(cmp) n=3	Relative ³ percentage (%)
WT	39,200±3,800	100
Nonfused NS5Bt	36,000± 3,200	91
E18A	1,100 ±100	2.5
E18D	800 ±100	2
H502A	900 ±110	2.5
H502K	1,200 ±110	3
H502R	900 ±110	2.5
E18H	2,000±20	5
H502E	1,800±200	4
E18H+H502E	28,000±1,000	70
G317V	1,200± 200	5
G317V+WT	38,200±3,900	100
Y276A+WT	37,480±3,800	95
GST	200 ± 40	0.5
GST+WT	38,000±4,000	100

¹ All experiments were independently done three times. The RdRP activity of wild GST-NS5Bt was taken as the standard.