Mutational analysis of the structure and functions of Hepatitis C Virus RNA-dependent RNA polymerase (RdRP)

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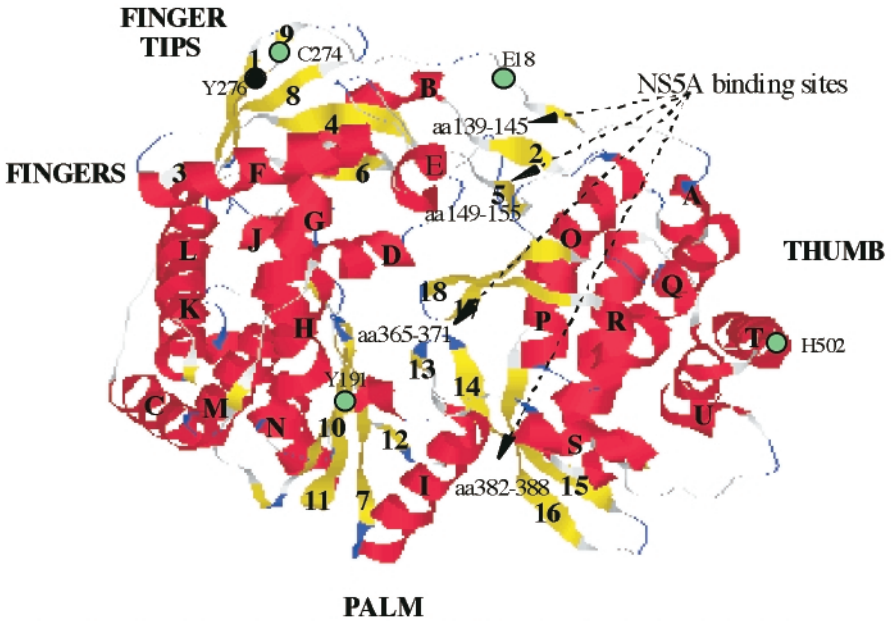
HCV NS5B is RdRP, a central catalytic enzyme for HCV replication. To further understand the structure and functions of NS5B, we introduced a series of 27 clustered and 19 point substitution mutations within and outside the ready-known motifs conserved among RdRP by alanine scanning and investigated effects of these mutants on putative properties of NS5B. The GST-fused form of NS5Bt, deleting the C-terminal membrane-anchoring domain, were bacterially expressed and purified as reported previously. Four clustered mutants, cm20t, cm194t, cm2t and cm3t, are defective in RdRP activity. By further analysis with point mutations within these regions, E18, Y191, C274, Y276, and H502, were found to be critical for the RdRP activity. By RNA filter binding assay, 3 sequences (aa 149-155, aa 220-226 and aa 276-280) were important for single strand RNA binding, but finally Y276 was the only residue essential for template/primer-binding. In light of the crystal structure models recently reported, our result indicated that the longer loop in the N-terminal region and the helix located at the top of thumb play important roles in the catalytic activity of RdRP of NS5B. These two substructures are unique among RdRPs and the other reverse transcriptases reported implies the uniqueness of HCV RdRP not only in structure but also in function.



The newly identified 5 residues essential for RdRP activity of NS5B.

The 5 residues (cicle in green or black color) are E18 at the long loop, Y191 at palm, C274, and Y276 at finger tip, and at thick thumb, respectively. Y276 (black circle9 is defective in template/primer-binding prerequisite to RdRP activity. E18 and H502 residues are far from the Catalytic pocket as reported (Qin et al., Hepatol., 2001).