Inter-domain interactions among dengue virus NS5 and NS3 are crucial for viral replication

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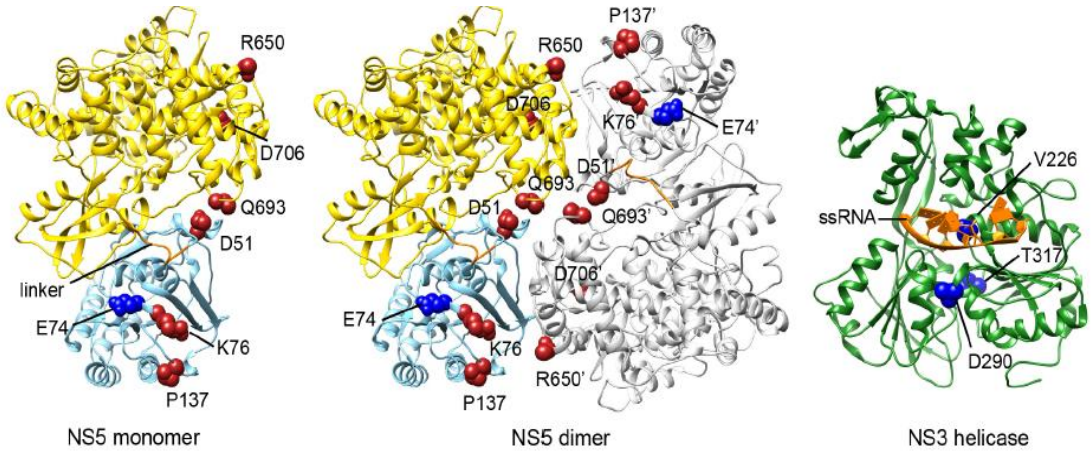
Four serotypes of mosquito-borne Dengue virus (DENV1-4), evolved from a common ancestor, are human pathogens of global significance, and there is no vaccine or antiviral drug available. The N-terminal domain of DENV NS5 has guanylyltransferase and methyltransferase (MTase) and the C-terminal region has the polymerase (POL) which are important for 5'- capping and RNA replication. The crystal structure of NS5 showed it as a dimer but the functional evidence for NS5 dimer is lacking. The results of our studies show that the substitution of DENV2 NS5 MTase or POL with that of DENV4 NS5 within DENV2 RNA resulted in severe attenuation of replication in the transfected BHK- 21 cells. A replication competent species evolved with acquired mutations in the DENV2 and DENV4 NS5 MTase or POL domain or in DENV2 NS3 helicase domain in the DENV2 chimera RNAs by repeated passaging of infected BHK-21 or mosquito cells. The linker region of seven residues in NS5, rich in serotype-specific residues, is important for recovery of replication fitness in the chimera RNA. Our results, taken together, provide genetic evidence for serotype-specific interaction between NS3 and NS5 as well as specific inter-domain interaction within NS5 required for RNA replication. Genome-wide RNAseq analysis revealed the distribution of adaptive mutations in RNA quasispecies. Those within NS3 and NS5 are located at the surface and/or within the NS5 dimer interface providing a functional significance to the crystal structure NS5 dimer.



The methyltransferase and RNA-dependent RNA polymerase domains of dengue virus NS5, and NS3 containing helicase and 5'-RNA triphosphatase in a complex, are required for 5'-capping and RNA replication. The crystal structure of NS5 dimer and the adaptive mutations accumulating at the two NS5 monomer interface and NS3 surface during replication of inter-serotypic NS5 chimera RNAs suggest that genome replication and 5'-capping are coupled. (adapted from Tadahisa Teramoto, Anuradha Balasubramanian, Kyung H. Choi, and Radhakrishnan Padmanabhan. J. Biol. Chem. 292(23) 9465-9479 (2017)