Functional Characterization of a Conserved RNA Structural Element in Dengue Virus Replication

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Dengue virus (DENV), a mosquito-borne virus in the *Flaviviridae* family, consists of four genetically distinct, yet closely related serotypes (DENV1-4). Over the past 20 years, DENV has increasingly become one of the worst mosquito-borne pathogens affecting humans, and its geographic distribution is expected to expand as changing climate conditions increase the range of mosquito vectors. Previous studies identified candidate RNA elements, both sequence-based and structural, that are heavily conserved and may regulate viral replication through interaction with viral proteins. Research into DENV replication strategies is critical for the development of antiviral medications and more effective vaccines, as no direct antiviral treatments have yet been approved for dengue fever. A candidate RNA structural element in the capsid-coding region of the DENV2 genome was previously identified using DENV1-4 genome alignments and secondary structure predictions, suggesting a potential role in viral replication. Two mutant variations (Mut1 and Mut2) of the candidate RNA structural element, the conserved capsid stem loop (CCSL), were cloned into an intermediate vector (pGEM-T). Generation of the CCSL Mut2 pGEM-T construct was confirmed using diagnostic restriction digest and agarose gel electrophoresis. Presence of the isolated CCSL Mut2 amplicon and cut wild type infectious clone plasmid (pD2/IC) was confirmed using subcloning restriction digests and agarose gel electrophoresis. Moving forward, mutant pD2/IC constructs will be used to generate mutant viral RNAs that will be analyzed using functional assays to examine how the mutations affect viral replication. These studies are crucial for the development of future antiviral treatments for combating dengue fever.