Mutagenic Analysis of Putative RNA Sequence Elements in the Dengue Virus Genome

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Dengue fever is caused by a mosquito-borne flavivirus, resulting in a debilitating and potentially deadly illness known as “breakbone fever”. The dengue virus (DENV) serogroup consists of four distinct but related viruses (DENV 1-4). Several RNA elements within the DENV genome are heavily conserved across DENV 1-4 and have been shown to be necessary for regulating viral replication through interactions with their specific sequence or their secondary structure. Research into viral replication strategies is essential for the development of effective antiviral medications, as there is currently no treatment for dengue fever outside of supportive care. Previously, candidate RNA sequence elements within the DENV genome were identified based on conservation across DENV 1-4, implying they may be important for viral replication. We began cloning mutant variations for two conserved RNA sequence elements within the DENV genome: Conserved Protease Coding-Region 1 (CPCR-1) and Methyltransferase RNA-Dependent Coding-Region 1 (MRdCR-1). Mutant amplicons were successfully amplified using overlap extension PCR mutagenesis and then subcloned into an intermediate vector (pGEM-T) to be propagated in competent *Escherichia coli*. Attempts to generate mutant pGEM-T constructs were unsuccessful, but once the presence of the desired mutations in pGEM-T is confirmed, mutant amplicons will be subcloned into a DENV2 infectious cloning vector (pD2/IC). Mutant pD2/IC constructs will be used to generate mutant viral RNAs that can be analyzed in functional assays to determine the effect of these mutations on viral replication and infectivity. This understanding will be foundational for the development of novel antiviral medications to combat dengue fever.