Progress and Prospects: Flavivirus RNA Replication

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Japanese encephalitis virus (JEV), a mosquito-borne flavivirus of the family *Flaviviridae*, is closely related to several clinically-important human pathogens, such as West Nile virus, yellow fever virus, dengue virus, St. Louis encephalitis virus, and Murray Valley encephalitis virus. JEV is a spherical enveloped virus, with a single-stranded positive-sense RNA genome of ~11,000 nucleotides (nt). The genome consists of a single long open reading frame (ORF) with two noncoding regions (NCRs) at the 5' and 3' ends. The ORF is initially synthesized into a large polyprotein, which is co- or post-translationally processed by host and viral proteases into at least ten mature proteins: (*i*) three structural proteins designated the capsid, premembrane, and envelope proteins and (*ii*) seven nonstructural proteins designated NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 proteins. The viral nonstructural proteins form a viral replicase complex that regulates the replication of the genomic RNA in the cytoplasm of the infected cell.

Although significant progress has been made in identifying *cis*-acting RNA elements within the 3'NCRs that are essential for RNA replication, most of these elements are limited to the ~100-nt 3'-terminal region that is highly conserved in these viruses. However, the functional importance of the remaining 5'-proximal region of the 3'NCR, which differs in sequence between the various serological groups, is poorly understood. In particular, sequence comparisons and computer modelings have suggested that in addition to the well-characterized ~100-nt 3'-proximal region, the remaining ~474-nt 5'-proximal region of the JEV 3'NCR also includes several RNA elements that may play an important role in JEV biology. However, the functional significance of these potential elements in JEV RNA replication has been remained to be demonstrated.

Recently, we have utilized an infectious cDNA technology to understand the roles in the virus life cycle of 3' *cis*-acting elements scattered throughout the 574-nt JEV 3'NCR. Our study has identified an array of 3' *cis*-acting RNA elements required for the complete replication of JEV genomic RNA. We have conducted a series of experiments involving (*i*) generation of genome-length mutant RNAs bearing a 5'- or 3'-truncated version of the 3'NCR, (*ii*) analysis of their phenotypes in susceptible BHK-21 cells after RNA transfection, and (*iii*) genotypic and phenotypic characterization of their progeny virions recovered from RNA-transfected cells. Our findings provide not only new information regarding 3' *cis*-acting elements, which play a critical role in controlling JEV RNA replication, but also offer new insights into 3' directed repeat sequences that are newly acquired by sequence duplication of the coding and 3' noncoding regions of JEV genomic RNA.