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A Complex RNA Motif: A Molecular Switch for Flavivirus Genome Replication?

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The positive-strand RNA viruses include a large number of important human, animal, and plant pathogens, and their replication is a critical element in the pathogenesis of a variety of acute and chronic diseases in humans, such as severe acute respiratory syndrome, encephalitis, and hepatitis and liver carcinogenesis. Although the precise details of their replication vary for each virus, diverse groups of positive-strand RNA viruses in plants and animals share remarkably common replication strategies. Following viral infection, positive-strand genomic RNA is released from endocytosed virions into the cytoplasm of the infected cell, initiating the synthesis of viral proteins, which then direct the replication of multiple positive-strand genomic RNA copies and their encapsidation into new progeny virions. The replication of genomic RNAs requires a complex of *cis*-acting RNA elements within the viral genome. These elements possess considerable secondary and tertiary structure that is specifically recognized by a complex of viral replicases and cellular proteins. Substantial progress has been made in identifying and characterizing the conserved primary sequences and secondary structures that are characteristic of *cis*-acting RNA elements in positive-strand RNA viruses; how these RNA elements participate in and coordinate the replication of the genomic RNA are, however, still challenging questions to be addressed. Therefore, in the present study we have analyzed a complex RNA motif within the genome of the Japanese encephalitis virus (JEV) that plays an essential regulatory role in RNA replication.

Tertiary or higher-order RNA motifs that regulate replication of positive-strand RNA viruses are as yet poorly understood. Using JEV, we now show that a key element in JEV RNA replication is a complex RNA motif that includes a string of three discontinuous complementary sequences (TDCS). The TDCS consists of three 5-nucleotide-long strands, the left (L) strand upstream of the translation initiator AUG

adjacent to the 5'-end of the genome, and the middle (M) and right (R) strands corresponding to the base of the flavivirus-conserved 3' stem-loop structure near the 3'-end of the RNA. The three strands are arranged in an antiparallel configuration, with two sets of base-pairing interactions creating L-M and M-R duplexes. Disrupting either or both of these duplex regions of TDCS completely abolished RNA replication, whereas reconstructing both duplex regions, albeit with mutated sequences, fully restored RNA replication. Modeling of replication-competent genomes recovered from a large pool of pseudorevertants originating from six replication-incompetent TDCS mutants suggests that both duplex base-pairing potentials of TDCS are required for RNA replication. In all cases, acquisition of novel sequences within the 3'M-R duplex facilitated a long-range RNA-RNA interaction of its 3'M strand with either the authentic 5'L strand or its alternative (invariably located upstream of the 5' initiator), thereby restoring replicability. We also found that a TDCS homolog is conserved in other flaviviruses. These data suggest that two duplex base-pairings defined by the TDCS not only play an essential regulatory role in a key step(s) of flavivirus RNA replication but also present a novel target for antiviral intervention.