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**A Complex RNA Motif That Is Essential for Japanese Encephalitis  
Virus Genome Replication**

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We show for the first time a complex RNA motif that consists of a string of three discontinuous complementary sequences (TDCS) that is essential for JEV RNA replication. Using hypothesis-driven systematic mutational analysis of the genomic RNA, recovery of a large pool of pseudorevertants bearing a variety of compensatory mutations, and identification and characterization of a number of novel sequences that are capable of restoring JEV RNA replication, we provide strong evidence that the TDCS is required for a formation of the intact 3'SL structure, long-distance communication between the 5' and 3' termini of the genome, and the complete replication of the genome. Multiple interactions involving both Watson-Crick and non-Watson-Crick base-pairs, rather than the specific nucleotide sequences of this motif *per se*, are critical not only for the formation and maintenance of a long-range RNA-RNA interaction but also for its biological functions. Thus, our data suggest that a complex RNA motif defined by the TDCS is a key regulatory component of JEV RNA replication, ensuring long-distance communication of the 3'SL with the 5'SL that precedes the translation initiation codon. This unique sequence-specific RNA motif offers an attractive target for prophylactic or therapeutic interventions that would block the replication of this clinically significant virus.