During the course of virus replication, the plus-sense RNA genome of Japanese encephalitis virus (JEV) is converted to a minus-sense template, which is subsequently used for the synthesis of a large number of plus-sense genomic RNA molecules. Since eukaryotic cells do not possess the RNA replication machinery, it is conceivable that the virus genome must code for it. A large number of proteins besides the DNA-dependent DNA polymerase are involved in DNA replication. It is therefore likely that several proteins besides the RNA-dependent RNA polymerase (RdRp) are involved in RNA replication. As RNA virus genome has limited coding capacity, it can be argued that some of the host cell proteins are made use of by the virus for its genome replication.

As the 5’- and the 3’-NCRs of flaviviruses have sequences and structures predicted to be involved in the replication of the viral genome, proteins that specifically bind these regions are likely to be involved in the process. Indeed, several host proteins have been described that bind the NCRs of flaviviruses as well as other RNA viruses, and roles of these proteins in RNA replication has been demonstrated in some cases. In the case of JEV, our lab had previously demonstrated that at least three mouse brain proteins of apparent molecular sizes of 32, 35 and 50 kDa interacted with the conserved 3’-terminal stem-loop (SL) structure of the 3’-NCR. One of these proteins was identified as the 36-kDa Mov34 protein. In the present work, the 50 kDa protein interacting with the 3’-SL was shown to be the La protein which also bound the 5’-NCR of JEV genome. The interaction of JEV RNA and La protein was demonstrated both in vitro as well as in vivo. The protein was shown to bind the loop regions in the NCRs through the N-terminal domain. As the C-terminal half of La protein contains a dimerization domain, binding of the N-terminal portion of La protein with JEV 3’- and 5’-NCR may circularize the genome for its efficient translation and replication.

The RNA-protein interaction studies were then done with recombinant human La protein (rhLa) that was shown to bind JEV 3’-SL and 5’-NCR sequences in a specific manner. The RNA-protein binding was demonstrated using electromobility shift assay, ultra-violet light-induced RNA-protein cross-linking, Northwestern blotting, and antibody-mediated super shift assays. The in vivo interaction of JEV RNA with La protein was demonstrated using the yeast three hybrid system and RNA-protein co-immunoprecipitation from JEV-infected human embryonic kidney 293A cells. The
Summary
dissociation constant ($K_d$) of the RNA-protein interaction was determined to be in the nanomolar range thereby indicating it to be physiologically possible. The sites of interaction of La protein with the JEV genome, characterized using toe printing-primer extension analysis, were found to be highly conserved RNA structures among flaviviruses at both the ends of the genome. The truncated versions of rhLa were used to demonstrate that the N-terminal half of the protein was involved in binding with JEV RNA and the C-terminal half of La protein containing the dimerization domain had no role in RNA binding.

La protein is a ~50 kDa protein that is highly abundant in eukaryotic cells. The protein interacts with a large variety of ligands and is the first protein that associates with all newly synthesized RNA polymerase III transcripts via their common UUU-OH 3' termini. La protein is largely confined to the nucleus though in certain circumstances, a fraction of La protein becomes cytoplasmic e.g. during Polio virus infection. The amino acid sequence alignment of La protein from various organisms reveals that these proteins can be divided into two domains. The highly conserved N-terminal domain and the less conserved C-terminal domain. The N-terminal half consists of RNA-recognition motifs 1 and 2 while the C-terminal half which is rich in basic amino acids contains another RNA-recognition motif and a protein dimerization domain.

La protein has been shown to bind the genomic RNA of several viruses and in some cases the consequences of this RNA-protein interaction have been elucidated. Thus, the protein was shown to be important for translation initiation and enhancement during Polio virus replication, or for alleviating the translation repression in the case of Human Immunodeficiency and Hepatitis C, and Encephalomyocarditis viruses. Further, La protein was shown to protect Hepatitis C virus RNA from degradation. The exact role of La binding to JEV 3'- and 5'-NCR is not clear at this stage but is likely to play a role in the circularization of the genome thereby facilitating the genome translation and transcription.