Introduction
Japanese Encephalitis virus (JEV) is a member of the *Flaviviridae* family of animal viruses, which includes several other medically important pathogens such as Yellow Fever (YF), Dengue (DEN) and West Nile (WN) viruses. JEV, a mosquito-borne virus, is the leading cause of viral encephalitis in humans. The virus is responsible for frequent epidemics of Japanese encephalitis (JE) in most parts of Southeast Asia, China and India. Approximately 50,000 cases of JE occur annually of which ~30% result in mortality and another 30% in long lasting neurological complications. Although there are no reliable figures available for the incidence of JE in India, up to 10,000 cases are believed to occur annually, making JE a serious medical problem in India and Asia (Kalita and Misra 1998; Misra et al. 1998; Tiroumourougane et al. 2002; Umenai et al. 1985). As a prophylactic measure, a mouse brain-derived JE vaccine is available, although in limited quantities. However, there is no virus-specific treatment available to help the JE patients. Understanding the virus replication at the molecular level may allow the development of potential inhibitors of virus replication.

The JEV genome consists of a single-stranded, positive-sense RNA of ~11 kb, having a type I cap with no poly adenylation at the 3'-end. The genomic RNA contains a single open reading frame (ORF) encoding a polyprotein of ~3400 amino acids. The polyprotein is subsequently cleaved by the viral and host proteases into three structural (capsid, pre-M and envelope) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) (Chambers et al. 1990a; Lindenbach and Rice 2001). The JEV ORF is flanked by 5'- and 3'-noncoding regions (NCRs) of 95 and 585 bases, respectively (Sumiyoshi et al. 1987; Vrati et al. 1999; Yun et al. 2003). Although the size and the sequence of the NCRs is not conserved well among different flaviviruses, several conserved features and secondary structures have been elucidated (Brinton and Dispoto 1988; Hahn et al. 1987; Proutski et al. 1997; Rauscher et al. 1997; Rice et al. 1985; Sumiyoshi et al. 1987; Sumiyoshi et al. 1992; Takegami et al. 1986). Thus for the 5'-NCR, secondary structure consisting of a stem with a small loop and a larger side loop has been predicted and confirmed by the secondary structure prediction programs, co-variation analysis and biochemical probing (Brinton and Dispoto 1988; Cahour et al. 1995; Hahn et al. 1987). Among different flaviviruses, these structures in the 5'-NCR are of same size, shape and thermodynamic stability. Similarly, the last 80-90 bases at the extreme 3' end of
the 3'-NCR have been predicted to form stable stem-loop (SL) structure among different flaviviruses (Brinton et al. 1986; Shi et al. 1996; Takegami et al. 1986). Even though the size and shape of the 3'-SL structure is highly conserved, sequence conservation is restricted to its loop regions and to 27 nucleotides immediately upstream of the structure. The conservation of the location and the structure of the 3'-SL in the 3'-NCRs of flaviviruses indicate its functional significance.

Following the infection, JEV and other flaviviruses induce proliferation and reorganization of cellular membranes, making structures like paracrystalline structures, convoluted membranes, and vesicle particles. The vesicle particles have been described to be the most likely site of viral RNA replication (Mackenzie et al. 1996; Mackenzie et al. 1998; Westaway et al. 1997; Westaway et al. 1999). These structures have been seen only during later time of infection and it is presently unclear where the early events in replication occur.

The mechanism followed by flaviviruses to replicate their genomes is not well understood. The flavivirus replication starts with the synthesis of complementary negative strand, thus forming a double stranded (ds) replicative form (RF) RNA, which then ultimately serves as the template for the production of additional positive-stranded genome via formation of a replication intermediate (RI). RI contains duplex of positive and negative strands where the positive strand is being displaced by the nascent strand undergoing elongation. RNA synthesis is asymmetric leading to accumulation of 10-100-fold excess of plus strand over the minus strand. (Chambers et al. 1990a; Chu and Westaway 1985; Cleaves et al. 1981; Uchil and Satchidanandam 2003).

The finer mechanism of RNA replication in virus-infected mammalian cells is not well understood although we know a great deal about DNA replication that requires a multitude of proteins besides the DNA polymerase. The RNA replication is, therefore, likely to require several proteins besides the RNA-dependent RNA polymerase which is encoded by the viral genome. Since RNA virus genomes have only limited coding capacity, it is possible that some of these proteins are of the host origin. The presence of NCRs in good proportion of the genome (~7%), when the genome is constrained for the coding capacity, points to their important role in the viral life cycle. The conserved
structures and sequences in the 5'- and 3'-NCRs despite sequence diversity indicates to their possible functional relevance in viral replication cycle. Thus, the NCRs might play role in minus strand synthesis, switch from minus to plus strand synthesis, initiation of translation of genome, and packaging of genome into the core of the virus during assembly. The 3'-NCR nucleotide sequence and its structural elements such as the 3'-SL structure are predicted to contain cis-acting signals for the viral RNA synthesis initiation, and these may, thus, interact with viral and cellular proteins involved in viral RNA replication (Chambers et al. 1990a; Chen et al. 1997; You and Padmanabhan 1999).

A number of cellular proteins have been shown to specifically interact with the NCRs of RNA viruses. For example, elongation factor-1α interacts with the 3'-SL structures of Dengue 4 (DEN4) and West Nile viruses (Blackwell and Brinton 1997; Nova-Ocampo et al. 2002), and the aminoacylated 3'-end of Turnip Yellow Mosaic virus RNA (Joshi et al. 1986). This protein also binds to the 5'-NCR of Polio virus RNA (Harris et al. 1994). Another protein calreticulin binds the 3'-NCR of DEN4 and Rubella genome (Singh et al. 1994; Yocupicio-Monroy et al. 2003). The polypyrimidine tract binding protein has been shown to interact with the 5'- and 3'-NCRs of Hepatitis C (Ito and Lai 1997; Tsuchihara et al. 1997) and Norwalk viruses (Gutierrez-Escolano et al. 2000; Gutierrez-Escolano et al. 2003). This protein also bound to the 3'-NCR of JEV (Kim and Jeong 2006) and DEN4 (Nova-Ocampo et al. 2002), and 5'-NCR of Mouse Hepatitis virus (Chang et al. 1994; Furuya and Lai 1993). Several other proteins, such as heterogeneous nuclear ribonucleoprotein (hnRNP) A1, hnRNP L, hnRNP C, HuR and poly (A)-binding protein, have been shown to bind the NCRs of different viral genomic RNAs (Hahn et al. 1987; Huang and Lai 2001; Shi et al. 2000; Zhang et al. 1998). Yet another protein La has been shown to interact with the NCRs of DEN4 (Garcia-Montalvo et al. 2004; Nova-Ocampo et al. 2002), Human Immunodeficiency (Chang et al. 1994), Sindbis (Pardigon and Strauss 1996), and Norwalk viruses (Gutierrez-Escolano et al. 2003).

Our laboratory has previously shown that the 3'-SL structure of JEV genome interacted with at least three cellular proteins of apparent molecular masses of 32, 35 and 50 kDa. One of these proteins was identified as the 36-kDa Mov34 protein (Ta and Vrati 2000). Studies described in this thesis were undertaken to identify other host proteins that
interact with the 3'SL of JEV genome. I have identified the 50-kDa protein to be the La protein. This protein is found abundantly in all cell types and is involved in various functions including the RNA stabilization and its transcription. My studies show that in vitro La protein binds both the 3'- and 5'-NCRs of JEV with high affinity. La protein was also shown to bind the JEV genome during the virus infection of mammalian cells. The La protein was shown to bind the NCR RNAs at the top loop structure via its N-terminal domain. The C-terminal domain contains a dimerization domain. The La protein binding to 3'- and 5'-NCRs may result in the circularization of genome leading to its efficient transcription and translation, and thereby efficient virus replication.