Introduction
Japanese encephalitis virus (JEV) belongs to *flaviviridae* family of animal viruses. The virus contains an ~11 kb single stranded, positive-sense RNA genome. The genome has type I cap at its 5’-end but lacks a poly(A) tail at its 3’-end. It serves as the only viral mRNA and encodes a single open reading frame of about 10 kb. A large polyprotein of ~3400 amino acids is translated from this open reading frame, which is both co- and post-translationally processed by cellular and viral proteases into three structural (Capsid, pre-Membrane and Envelope) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). The coding region of the genome is flanked by 5’- and 3’-non-coding regions (NCRs) that are 95 and 585 nucleotides long, respectively (Sumiyoshi *et al.*, 1987; Vrati *et al.*, 1999).

The mechanism followed by the flaviviruses to replicate their genomes is not well understood. Subsequent to the entry of the genome into the host cell, it undergoes translation to produce a long polyprotein, which is then cleaved into structural and non-structural proteins. Some of these non-structural proteins are involved in the assembly of the RNA replicase complex, which then binds the 3’-end of the genome to synthesize a minus-sense RNA (or the antigenome) as the replication intermediate (Rosa Martha *et al.*, 2003). The antigenome is then used as a template for the synthesis of a large number of plus-sense genomic RNA molecules. Thus, replication would require RNA transcription from the 3’-ends of both the genomic as well as the antigenomic RNA. Before the onset of replication, translation of the genome to produce proteins necessary for virus replication would require interaction of cellular proteins with the 5’-end of the genomic RNA. Thus both the 3’- and the 5’-ends of the genomic RNA and the 3’-end of the antigenome are important for the virus RNA replication. The presence of conserved secondary structures within both the NCRs suggests that they might contain *cis*-acting elements involved in translation and replication.

The composition of the flavivirus RNA replication complex is not clear though there is evidence for the involvement of the non-structural proteins NS5, which is the RNA-dependent RNA polymerase (RdRp) of the virus, and NS3
during JEV replication (Uchil and Satchidanadam, 2003). A great deal of information is available for DNA replication that requires several proteins besides the DNA polymerase. It could, therefore, be extrapolated that RNA replication would require proteins other than the RdRp. RNA viruses usually have smaller genomes with only limited protein coding capacity and therefore they must rely on existing or modified cellular machineries for many steps of RNA synthesis. Indeed, there is increasing evidence that RNA viruses frequently subvert cellular proteins for replication and transcription of viral RNAs.

The participation of cellular proteins in viral RNA-dependant RNA synthesis follows two modes. In the first, cellular proteins form a part of the RdRp holoenzyme. A number of host proteins have been shown to be associated with RdRp of phage Qβ (Bluementhal and Carmichael, 1979), Brome mosaic (Quadt et al., 1993), Cucumber mosaic (Hayes and Buck, 1990), Tobacco mosaic (Osman and Buck, 1997), Vesicular stomatitis (Das et al., 1998), Measles (Moyer et al., 1990), Influenza (O’Neill and Palese, 1994) and Polio viruses (Harris et al., 1994, McBride et al., 1996). In the second mode, the cellular proteins bind directly to the RNA template, thus directing RdRp to the template or localizing the template RNA to a microenvironment suitable for its replication. A variety of host proteins has been shown to interact with putative cis-acting elements of Sindbis (Paradigon and Strauss, 1996), Mouse hepatitis (Li et al., 1997), Hepatitis C (Ito and Lai, 1997; Ali and Siddiqui, 1997; Tsuchihara et al., 1997), Rubella (Singh et al., 1994 Pogue et al., 1996) Human immunodeficiency (Chang et al., 1994; Svitkin et al., 1994; Black et al., 1996) and other RNA viruses (reviewed in Lai, 1998). In Potato X virus and Mouse hepatitis virus host protein binding sites on viral RNAs were shown to co-localise with cis-acting sequences that were demonstrated to be required for viral replication (Sriskanda et al., 1996; Yu and Leibowitz, 1995). These studies suggested that the interaction between host proteins and viral cis-acting elements might be functionally relevant for the replication of viral RNA.
As for flaviviruses, Blackwell and Brinton (1997) have described the binding of translation elongation factor-1α (EF-1α) with the 3'-NCR of West Nile virus (WNV). Brinton’s group has also described binding of TIA-1 and TIAR with the 3'-end of the WNV antigenome (Li et al., 2002). Three proteins from mouse brain were shown to interact with the 3'-NCR of the JEV genome by our laboratory (Ta and Vrati, 2000). One of these proteins was identified as Mov34 protein that may have a role in RNA transcription.

With a view to understand JEV replication, studies were undertaken to identify and characterize cellular proteins from mouse brain that interact specifically with JEV RNA sequences likely to be involved in the genome replication. In vitro synthesized radiolabelled RNA representing the 5'- and the 3'-NCRs of JEV genome and the 3'-end of the antigenome were used to identify cellular proteins from neonatal mouse brain that specifically interacted with them. Using gel mobility shift assays, UV-cross-linking of RNA and proteins, Northern blotting and cDNA library screening with RNA probes, I have identified the 240-kDa alpha II spectrin and 12-kDa ATPase inhibitor proteins as binding to the 5'-NCR of JEV and an 80-kDa SV2A protein as binding to the 3'-NCR of JEV. These studies are reported in this thesis and implications of the interaction of these proteins with JEV genome for its replication and virus pathogenesis are presented.