Hepatitis C virus (HCV) is a positive sense, single stranded RNA virus belonging to the genus *Hepacivirus* and the family *Flaviviridae*. It infects human liver cells predominantly. Although, the treatment with α interferon and ribavirin can control HCV in some cases, they fail to achieve sustained virological response in others, thus emphasizing the need of novel therapeutic targets.

The viral genome is 9.6 kb long consisting of a 5’ untranslated region (5’UTR), a long open reading frame (ORF) that encodes the viral proteins and the 3’ untranslated region (3’UTR). The 5’UTR contains a *cis* acting element, the internal ribosome entry site (IRES) that mediates the internal initiation of translation. The HCV 5’UTR is highly structured and consists of four major stem-loops (SL) and a pseudoknot structure. HCV proteins are synthesized by the IRES mediated translation of the viral RNA, which is the initial obligatory step after infection. The viral proteins are synthesized in the form of a long continuous chain of proteins, the polyprotein, which is then processed by the host cell and the viral proteases. Once viral proteins are synthesized sufficiently, the viral RNA is replicated. However the mechanism of switch from translation to viral RNA replication is not well understood. Several host proteins as well as the viral proteins help in the completion of various steps in the HCV life cycle. In this thesis, the role of two such factors in HCV RNA translation and replication has been characterized and exploited to develop anti-HCV peptides.
The HCV proteins are categorized into two major classes based on the functions broadly: the non structural and the structural proteins. HCV NS3 protein (one of the viral non structural proteins) plays a central role in viral polyprotein processing and RNA replication. In the first part of the thesis, it has been demonstrated that the NS3 protease (NS3\textsuperscript{pro}) domain alone can specifically bind to HCV-IRES RNA, predominantly in the SLIV region. The cleavage activity of the NS3 protease domain is reduced upon HCV-RNA binding owing to the participation of the catalytic triad residue (Ser 139) in this RNA protein interaction. More importantly, NS3\textsuperscript{pro} binding to the SLIV region hinders the interaction of La protein, a cellular IRES-trans acting factor required for HCV IRES-mediated translation, thus resulting in the inhibition of HCV-IRES activity. Moreover excess La protein could rescue the inhibition caused by the NS3 protease. Additionally it was observed that the NS3 protease and human La protein could out-compete each other for binding to the HCV SL IV region indicating that these two proteins share the binding region near the initiator AUG which was further confirmed using RNase T1 foot printing assay. Although an over expression of NS3\textsuperscript{pro} as well as the full length NS3 protein decreased the level of HCV IRES mediated translation in the cells, replication of HCV RNA was enhanced significantly. These observations suggested that the NS3\textsuperscript{pro} binding to HCV IRES reduces translation in favour of RNA replication. The competition between the host factor (La) and the viral protein (NS3) for binding to HCV IRES might contribute in the regulation of the molecular switch from translation to replication of HCV.

In the second part the interaction of NS3 protease and HCV IRES has been elucidated in detail and the insights obtained were used to target HCV RNA function. Computational approach was used to predict the putative amino acid residues within the protease that might be involved in the interaction with the HCV IRES. Based on
the predictions a 30-mer peptide (NS3proC-30) was designed from the RNA binding region. This peptide retained the RNA binding ability and also inhibited IRES mediated translation. The NS3proC-30 peptide was further shortened to 15-mer length (NS3proC-C15) and demonstrated ex vivo its ability to inhibit translation as well as replication. Additionally, its activity was tested in vivo in a mice model by encapsulating the peptide in Sendai virus based virosome followed by preferential delivery in mice liver. This virosome derived from Sendai virus F protein has terminal galactose moiety that interacts with the asialoglycoprotein receptor on the hepatocytes leading to membrane fusion and release of contents inside the cell. Results suggested that this peptide can be used as a potent anti-HCV agent.

It has been shown earlier from our laboratory, that La protein interacts with HCV-IRES near initiator AUG at GCAC motif by its central RNA recognition motif, the RRM2 (residues 112-184). A 24 mer peptide derived from this RRM2 of La (LaR2C) retained RNA binding ability and inhibited HCV RNA translation. NMR spectroscopy of the HCV-IRES bound peptide complex revealed putative contact points, mutations at which showed reduced RNA binding and translation inhibitory activity. The residues responsible for RNA recognition were found to form a turn in the RRM2 structure. A 7-mer peptide (LaR2C-N7) comprising this turn showed significant translation inhibitory activity. The bound structure of the peptide inferred from transferred NOE (Nuclear Overhauser Effect) experiments suggested it to be a β-turn. Interestingly, addition of hexa-arginine tag enabled the peptide to enter Huh7 cells and showed inhibition HCV-IRES function. More importantly, the peptide significantly inhibited replication of HCVRNA. Smaller forms of this peptide however failed to show significant inhibition of HCV RNA functions suggesting that the 7-mer
peptide as the smallest but efficient anti-HCV peptide from the second RNA recognition motif of the human La protein.

Further, combinations of the LaR2C-N7 and NS3\textsuperscript{pro}C-C15 peptide showed better inhibitory activity. Both the peptides were found to be interacting at similar regions of SLIV around the initiator AUG. The two approaches have the potential to block the HCV RNA-directed translation by targeting the host factor and a viral protein, and thus can be tried in combination as a multi drug approach to combat HCV infection.

Taken together, the study reveals important insights about the complex regulation of the HCV RNA translation and replication by the host protein La and viral NS3 protein. The interaction of the NS3 protein with the SLIV of HCV IRES leads to dislodging of the human La protein to inhibit the translation in favour of the RNA replication. These two proteins thus act as the regulators of the translation and the replication of viral RNA. The peptides derived from these regulators in turn regulate the functions of these proteins and inhibit the HCV RNA functions.