Hepatitis C is a major world health problem, caused by the Hepatitis C virus (HCV). Therapeutic intervention against HCV has been hindered by a lack of understanding of the fundamental processes in the viral life cycle. HCV uses a novel mechanism of translation initiation of its polyprotein mRNA. Instead of normal cap-mediated initiation, the 40S ribosomal subunit binds directly to an internal ribosome entry site (IRES) that is formed by the mRNA structure. This binding event tethers the 40S subunit near the start codon for translation. We have used a combined biochemical and biophysical approach to determine the structural basis for IRES-40S interaction and subsequent initiation. The HCV IRES is a complex mRNA structure of about 300 nts. We identified domains within the IRES that are involved in 40S subunit interaction using chemical probing. Mutations within these domains disrupt high IRES-40S subunit affinity ($K_d = nM$), as measured by native gel electrophoresis. Likewise, many mutations in these domains affect IRES activity, as measured in vivo in a dual luciferase translation assay. We have identified the protein components in the 40S subunit that may be required for IRES interaction using photocrosslinking.

The structures of RNA domains required for IRES-40S subunit interaction have been determined by NMR spectroscopy. Four stem-loop structures have been solved: domains IIb, IIId, IIIe and IV. The domains IIb and IIId loops have "Loop E" motif internal loops, with non-canonical G-A, A-A and U-A pairs, as well as base triples and local backbone inversions. These loop E motifs have been shown to be important for RNA-RNA and RNA-protein interaction, and are conserved among related pestiviruses. The domain IIIe loop structure is a novel tetraloop, whereas domain IV, which contains the start codon, is a flexible hairpin loop. We are currently investigating the structure of the intact, 90 kDa IRES RNA. We are building on assignments made from isolated oligonucleotide fragments, and using segmental labeling by RNA ligation. Our data indicate that the isolated stem loops form similar structures in the intact IRES.

Our results show how NMR, used in conjunction with biochemical and physical methods can provide powerful insights into RNA function.