

## Abstract

Translation initiation in the hepatitis C virus (HCV) occurs through a cap-independent mechanism that involves an internal ribosome entry site (IRES) capable of interaction with and utilization of the eukaryotic translational machinery. We focused on the structural configuration of the different HCV-IRES domains and the impact of IRES primary sequence variations on secondary structure conservation and function. For this purpose we introduced into our laboratory, methods such as denaturing gradient and temperature gradient gel electrophoresis for screening the degree of heterogeneity and total amount of HCV-IRES variability accumulated in HCV infected patients over a period of time. The selected samples showed variable migration pattern of the HCV-IRES (from all the patients) visualized in DGGE and TGGE, were sequenced and evaluated for translation efficiency using flow cytometry. In some cases, we discovered that multiple mutations, even those scattered across different domains of HCV-IRES, led to restoration of the HCV-IRES translational activity, although the individual occurrences of these mutations were found to be deleterious. We propose that such observation may be attributed to probable long-range inter- and/or intra-domain functional interactions. We established a large-scale HCV-IRES variation database (HCVIVdb; [www.hcvivbd.org](http://www.hcvivbd.org)) comprises ~1900 mutations acquired from majority of the HCV-IRES mutation-linked studies. The HCVIVdb contributes extensively by providing comprehensive HCV-IRES mutation dataset that can be utilized in overall evaluation of the functional role(s) played by structural IRES elements in modulation of HCV-IRES translation initiation. The design of the knowledge base web interface and advanced search tools is conducive to perform and visualize multiple analyses by comparisons of the collated HCVIVdb data, leading to new findings. We also identified ~20 novel HCV-IRES mutations and determined their influence on HCV-IRES translation efficiency by flow cytometry. Further elucidation of these novel mutations in the context of probable HCV-IRES structure conformations and contacts with translation machinery was demonstrated using the available mutation (HCVIVdb), structure and biochemical data. For validation of our HCVIVdb dataset we used multiple sequence alignment of the HCV genome data from other resources. These validations showed a positive correlation and signified the conservation of specific nucleotides and hypervariability of others. The extended data of the nucleotide variability also provides wider knowledge about

the evolutionary advantage and preservation of specific bases at each HCV-IRES nucleotide position. The structural conformation, sequence preservation and variability, and translational machinery association with the HCV-IRES regions are also thoroughly discussed, along with other factors that can affect and influence the formation of translation initiation complexes.

Moreover, our results indicate interplay between codon bias and mRNA secondary structure as determinants of translation efficiency of the HCV-core RNA during HCV-IRES mediated translation initiation.

**Keywords:** Hepatitis C virus, HCV, internal ribosome entry site, IRES, database, translation efficiency, structure, quasispecies, codon bias, core.