Charles University in Prague, Faculty of Science
Department of Genetics and Microbiology

Ph.D. study program:
Molecular and Cellular Biology, Genetics and Virology

Summary of the Ph.D. Thesis

A study of the HCV IRES variability: An experimental approach coupled with design of a large-scale mutation database.

Mgr. Anas Khawaja

Laboratory of RNA Biochemistry
Department of Genetics and Microbiology

Supervisor: RNDr. Martin Pospíšek, Ph.D.
Supervisor-consultant: Mgr. Václav Vopálenský, Ph.D.

Prague, 2016

Doctoral studies in biomedicine

Charles University in Prague, Faculty of Science,
Department of Genetics and Microbiology.

Program: Molecular and Cellular Biology, Genetics and Virology

Committe chair: Prof. RNDr. Stanislav Zadražil, DrSc.

Author: Mgr. Anas Khawaja

Supervisor: RNDr. Martin Pospíšek, Ph.D.

Supervisor-consultant: Mgr. Václav Vopálenský, Ph.D.

The full text of the thesis is available in the respective library of the Faculty of Science, Charles University in Prague.

Contents

List of abbreviations

aa	aminno acid
DGGE	denaturing gradient gel electrophoresis
DNA	deoxyribonucleic acid
EGFP	enhanced green fluorescent protein
eIF	eukaryotic translation initiation factor
HCV	hepatitis C virus
HCVIVdb	HCV-IRES variation database
IFN	interferon
IRES	internal ribosome entry site
NIAID	National Institute of Allergy and Infectious Diseases
nt.	nucleotide
NVR	non-sustained viral responders
ORF	open reading frame
RNA	ribonucleic acid
SVR	sustained viral responders
tAI	transfer RNA adaptation index
TGGE	temperature gradient gel electrophoresis
UTR	untranslated region
ViPR	Virus Pathogen Database and Analysis Resource

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) 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.

1. Introduction

Hepatitis C virus (HCV) is a blood-borne pathogen with an estimate of 170 million people as chronic carriers of the virus

(Gravitz, 2011). HCV is a positive sense single stranded RNA virus of the *Flaviviridae* family. The HCV genome has a 5' untranslated region (UTR), a polyprotein encoding region and a 3' UTR. The polyprotein is further co- and post-translationally processed to yield structural and non-structural proteins (Kato et al., 1990). The viral mRNA translation of hepatitis C virus (HCV) is one of the early steps in HCV life cycle mediated by an internal ribosome entry site (IRES), present in the 5' UTR (Honda et al., 1996). The HCV-IRES cap-independent mechanism of translation initiation involves direct positioning of 40S to the AUG start codon and use only a subset of canonical initiation factors (Pestova et al., 1998, Ji et al., 2004). The structural elements of IRES-RNA are highly conserved comprises domains (I-IV) that constitutes precise interaction(s) with ribosome subunits to establish stable binding of eIFs on HCV-IRES/40S complex, facilitating the assembly of the pre-initiation 80S translation complex (Locker et al., 2007, Tsukiyamakohara et al., 1992, Brown et al., 1992). Recently, with the aid of cryo-EM it has been shown that the structural rearrangements of HCV-IRES/40S necessitate the translation initiation (Quade et al., 2015, Yamamoto et al., 2015, Yamamoto et al., 2014). Additionally, the appropriate functioning of the HCV-IRES domains require the specificity and sequence/structure conservation of its domains II-IV and nucleotide changes in HCV-IRES can cause defect in viral mRNA translation (Manuscript 1) (Sizova et al., 1998, Collier et al., 2002, Jubin et al., 2000, Berry et al., 2011, Filbin and Kieft, 2011, Angulo et al., 2016, Bhat et al., 2015).

Here, we focused on the diversity of HCV-IRES and the overall effect it can have on viral protein synthesis. The presence of genetic variants in viral population(s) that has accumulated over a period of time in HCV infected patients was estimated by denaturing and

temperature gradient gel electrophoresis (DGGE and TGGE) that facilitated the characterization of HCV-IRES genetic heterogeneity. The functional scope of selected HCV-IRES variants from patients' samples were determined which prompted us to develop a comprehensive resource termed HCV-IRES variation database (HCVIVdb) based on the published data (Manuscript 2). The HCVIVdb permitted us to elucidate the range of HCV-IRES variant capabilities on a much wider-scale along with the insight into distinct characteristic of the HCV-IRES that can be attributed to a probable long-range inter or intra-domain interaction (Manuscripts 1 and 3). We demonstrated the impact of ~20 novel mutations on HCV-IRES through comparative analyses of mutation and biochemical data together with establishing the link of mutations' type and occurrence with the outcome of therapy. However, no direct link was found. The HCVIVdb was validated through HCV genome data from other resources and were found to be positively correlated, which lead to other interesting finds in context of the HCV-IRES hypervariability and conservation of specific nucleotides. We also demonstrated that codon bias and mRNA structure acts as determinants of translation efficiency in different HCV-core constructs through introduction of synonymous mutations having variable codon bias and mRNA structure.

The outcome of the thesis is 3 manuscripts and a large-scale HCVIV database (HCVIVdb: www.hcvivdb.org).

2. Aims of the study

The HCV mutates rapidly due to the lack of proof-reading polymerase generating genetically distinct and closely-related variants in individuals termed quasispecies. We initiated our research:

1. By aiming to design methods for screening of the HCV-IRES genetic heterogeneity in HCV infected patients, which would permit us to locate, visualize and measure the extent of variation(s) that may have accumulated in HCV-IRES over a period of time in HCV infected individuals. Hence, we develop a procedure that would monitor the amount of changes in virus population(s) corresponding to the duration and/or the effect on treated or non-treated patients by IFN-α and ribavirin therapy.

2. To measure the translation efficiency of selected HCV-IRES variants from the HCV infected patients in order to estimate the manner by which type, location and diversity of nucleotide change(s) influence the viral protein synthesis.

3. To achieve an over-all view of HCV-IRES mutation(s) capacity/impact on viral mRNA translation by collection and assembly of mutations from published data and compare it with the acquired patients' mutation dataset. A comprehensive insight into the behavior of HCV-IRES that could further lead to an understanding of the contribution made by specific IRES regions with regards to interaction with translation machinery, sequence conservation and maintenance of structural elements.

4. To establish a link of mutations' dispersion in different domains of the HCV-IRES to sustained and non-sustained HCV responders, treated and non-treated by standard therapy of interferon-α (IFN) and ribavirin. This would help us to identify the regions of mutations' occurrence and type of mutations that could lead to a sustained and non-sustained viral response, respectively.

5. To find out if the interplay between codon bias and mRNA secondary structure impacts the translation efficiency of HCV-core RNA during HCV-IRES mediated translation initiation. We chose

N-terminal region of the HCV-core gene to discover the effects of codon bias and mRNA structure as determinants of translation efficiency.

3. Material and methods

I used common methods of molecular biology and microbiology (DNA cloning, PCR, RNA and DNA analyses, western blots). For estimating the genetic heterogeneity among viral population(s) I used denaturing gradient (DGGE) and temperature gradient gel electrophoresis (TGGE). The transient expression of enhanced green fluorescent protein (EGFP) for testing of the HCV-IRES activity was measured using flow cytometry. The design of the HCV-core gene constructs for different codon bias was carried out using transfer RNA adaptation index (tAI) method as a measure of codon bias (Tuller et al., 2010). The mammalian HeLa [Chang Liver] ATCC ® CCL-13™ (aka CCL13) cell line was utilized for transient expression of the HCV-core constructs and the protein products were detected by western blotting and quantified using Carestream MI SE software (Kodak).

4. Results and discussion

Understanding the potential of HCV-IRES RNA structural elements in mediation of translation initiation was of particular interest of our research. In this study, we characterized the genetic variability of HCV-IRES by screening the viral IRES heterogeneity accumulated over a period of time in HCV infected patients using DGGE and TGGE methodology. An overview of all-around HCV-IRES variability among patients was observed. The selected samples from the patients were sequenced and measured for the

HCV-IRES translation efficiency by flow cytometry. A correlation was found between the increasing number of mutations present in a sample with low translation efficiency. However, the location of mutations' occurrence was found to be more crucial in specifying the outcome of HCV-IRES translation response. In an attempt to further gain insight into the behavior of HCV-IRES observed phenotypes from our patients' samples on a wider scale, we collected mutations from significant number of HCV-IRES mutation-related studies. The collection of ~1900 mutations was assembled into a resource called HCV-IRES variation database (HCVIVdb; www.hcvivdb.org). The data were collated into various categories that provide knowledge such as frequency of variations, measured activity in a translation assay, clinical data and original publication reference with additional/relevant information of the corresponding publication. The analytical tools available on the webpage permit immediate access to the variation entries classified to domains and sub-domains (10 categories), translation activity (5 categories) and genotype (5 categories). The HCVIVdb comprise tremendous amount of HCV-IRES specialized dataset allowed us to perform multiple comparative and functional analyses that demonstrated a large overview of all the HCV-IRES mutations and the observed phenotype(s), along with some unique characteristics of the IRES-RNA in maintaining the translation efficiency (Manuscript 2). The particular characteristic that we found can be accredited to a probable long-range inter- or intra-domain interaction, consisting of samples with multiple mutations the individual presence of which had been found to abrogate the HCV-IRES translation mediation. However, the collective occurrence of these individual mutations has shown to restore the viral mRNA translation. A reverse phenomenon with multiple mutations

presented in HCV-IRES causes a sharp decrease in activity whereas individual occurrence of these mutations was not detrimental (Manuscripts 1 and 3). From HCVIVdb dataset, we divided all the HCV-IRES mutations with reported impact on translation response in to two groups. The group 1 comprises all the individual mutations in HCV-IRES domains whereas the group 2 consists of multiple mutations. We calculated the cumulative average of HCV-IRES translation efficiency of group1 and 2, respectively and by doing so we identified HCV-IRES regions with higher vulnerability to mutations. The results of analyses of these two groups are in correspondence with each other, illustrating that different domains and sub-domains of the HCV-IRES, in both the groups, display essentially a similar effect on HCV-IRES mediated translation initiation. With the available HCVIVdb data, we mapped the naturally occurring HCV-IRES mutations on secondary structure from the HCV infected patients, to determine any correlation between the type/location of mutations which are possibly initiated by the antiviral therapy, and induced sustained and non-sustained viral response. The dispersion and frequency of mutations were shown to be random and overlap in both the populations of patients and no direct link could be established. We also identified the translation efficiency of ~20 novel HCV-IRES mutations and further established their functional roles in the context of sequence preservation and interaction with translation machinery through comparative analyses of the available mutation, structure and biochemical data (Manuscript 3).

The HCVIVdb was validated by multiple alignment of 2006 HCV genome sequence entries obtained from National Institute of Allergy and Infectious Diseases (NIAID) Virus Pathogen Database and Analysis Resource (ViPR) online through the web site at

http://www.viprbrc.org (Pickett et al., 2012) using MUSCLE software (Edgar, 2004, Edgar and Batzoglou, 2006). Both the datasets were plotted to find the possible variation from the consensus sequence of the HCV-IRES. The nucleotide count (nt. 1-356, which constitutes the HCV-IRES) from both the databases displayed hypervariablity of some particular HCV-IRES nucleotides and conservation of other nucleotides. Furthermore, the extended data of the nucleotide variability showed details in variation among bases from the consensus sequence at each HCV-IRES nucleotide position, which can reflect in understanding the evolutionary dynamics and genetic variations in HCV-IRES. The HCVIVdb is a unique central resource that provides specialized data related to HCV-IRES mutations and corresponding functional output with further classification which would allow the users to carry out multiple analyses, evaluate, manipulate and download the data.
In general, the results on HCV-IRES variability offer a detailed insight into the overall functional dynamics of HCV-IRES on the level of its domains, subdomains and individual nucleotides.

We also focused on the role of codon bias and mRNA structure in determining the translation efficiency of the HCV-core polypeptide. The HCV-core gene comprises highly structured RNA element with multiple stem-loops (Vassilaki et al., 2008). We employed truncated N-terminal region of HCV-core gene (58 amino acids, highly basic (Boulant et al., 2005)) to measure the interplay between codon bias and secondary structure during HCV-IRES mediated translation initiation. The codon bias was measured using transfer RNA adaptation index (tAI) (Tuller et al., 2010). The synonymous mutations were introduced in HCV-core constructs having differences in their codon usage and mRNA secondary structure and were evaluated for any changes in the gene expression

pattern. We found that both the codon bias and mRNA folding influence the translation efficiency of HCV-core RNA. Although, initial experiments exhibit interplay between the two measures, more experiments are required to reinforce the hypothesis.

5. Conclusions

1. We established tools (DGGE and TGGE) to measure and characterize the genetic variability of HCV-IRES in HCV infected patients, accumulated over a period of time.

2. With the aid of bicistronic pRG vector, the HCV-IRES activity of seleceted samples was measured by analyzing the transient expression of EGFP reporter gene in mammalian cell culture, employing flow cytometry. The expression pattern exhibits range of activities for different constructs.

3. A comprehensive HCV-IRES variation database (HCVIVdb: www.hcvivdb.org) was established that comprises mutation data from bulk of the HCV-IRES associated studies. The HCVIVdb contains ~1900 mutations collated into different classifications permitting the users to browse through the data, analyze, manipulate and download.

4. In the context of comparative analysis of available HCVIVdb data, identical mutations reported in separate studies sometimes showed differences in HCV-IRES translation efficiency.

5. An interesting and unique HCV-IRES feature was found which can be responsible for a potential long-range inter or intra-domain interaction culminating to translation efficiency restoration in multiple mutants. The individual occurrence of mutations from

these multiple mutants abrogates the HCV-IRES translation response.

6. The novel HCV-IRES mutations (~20) were found and their functional roles were established using flow cytometry and also in the context of their location, type, structure and interaction with the translation machinery.

7. The naturally occurring HCV-IRES mutations from the HCV infected patients, from published studies, were mapped on HCV-IRES secondary structure to establish a link between (a) frequency and location of mutations and (b) the probable outcome of HCV-therapy in sustained and non-sustained viral responders. No direct link could be found.

8. The HCVIVdb dataset was validated by comparing it with other resources. A positive correlation of these two datasets was found.

9. Variability of nucleotides of the two datasets (HCVIVdb and ViPR (integrated) resource) showed similarity, displaying hypervariability and conservation of particular nucleotides. It also offers deeper understanding of overall preservation / evolutionary advantage of specific HCV-IRES bases over the others.

10. HCVIVdb dataset comprises specialized information providing a central resource for researchers in the IRES and hepatitis C virus oriented fields. The HCVIVdb was compared with other viral, IRES and RNA databases to establish the distinction of our database and its contribution towards the field of HCV.

11. We also found that codon bias and mRNA structure contributes to the translation efficiency of HCV-core gene. The results showed interplay between codon bias and mRNA secondary structure in

shaping the translation efficiency of the HCV-core RNA during HCV-IRES mediated translation initiation.

6. References

ANGULO, J., ULRYCK, N., DEFORGES, J., CHAMOND, N., LOPEZ-LASTRA, M., MASQUIDA, B. & SARGUEIL, B. 2016. LOOP IIId of the HCV IRES is essential for the structural rearrangement of the 40S-HCV IRES complex. *Nucleic Acids Research,* 44**,** 1309-25.

BERRY, K. E., WAGHRAY, S., MORTIMER, S. A., BAI, Y. & DOUDNA, J. A. 2011. Crystal Structure of the HCV IRES Central Domain Reveals Strategy for Start-Codon Positioning. *Structure,* 19**,** 1456-1466.

BHAT, P., SHWETHA, S., SHARMA, D. K., JOSEPH, A. P., SRINIVASAN, N. & DAS, S. 2015. The beta hairpin structure within ribosomal protein S5 mediates interplay between domains II and IV and regulates HCV IRES function. *Nucleic Acids Research,* 43**,** 2888-901.

BOULANT, S., VANBELLE, C., EBEL, C., PENIN, F. & LAVERGNE, J. P. 2005. Hepatitis C virus core protein is a dimeric alpha-helical protein exhibiting membrane protein features. *Journal of Virology,* 79**,** 11353-65.

BROWN, E. A., ZHANG, H. C., PING, L. H. & LEMON, S. M. 1992. Secondary Structure of the 5' Nontranslated Regions of Hepatitis-C Virus and Pestivirus Genomic Rnas. *Nucleic Acids Research,* 20**,** 5041-5045.

COLLIER, A. J., GALLEGO, J., KLINCK, R., COLE, P. T., HARRIS, S. J., HARRISON, G. P., ABOUL-ELA, F., VARANI, G. & WALKER, S. 2002. A conserved RNA structure within the HCVIRES eIF3-binding site. *Nature Structural Biology,* 9**,** 375-380.

EDGAR, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research,* 32**,** 1792-1797.

EDGAR, R. C. & BATZOGLOU, S. 2006. Multiple sequence alignment. *Current Opinion in Structural Biology,* 16**,** 368-373.

FILBIN, M. E. & KIEFT, J. S. 2011. HCV IRES domain IIb affects the configuration of coding RNA in the 40S subunit's decoding groove. *RNA-a Publication of the RNA Society,* 17**,** 1258-1273.

GRAVITZ, L. 2011. A smouldering public-health crisis. *Nature,* 474**,** S2-S4.

HONDA, M., BROWN, E. A. & LEMON, S. M. 1996. Stability of a stem-loop involving the initiator AUG controls the efficiency of internal initiation of translation on hepatitis C virus RNA. *RNA-a Publication of the RNA Society,* 2**,** 955-968.

JI, H., FRASER, C. S., YU, Y. H., LEARY, J. & DOUDNA, J. A. 2004. Coordinated assembly of human translation initiation complexes by the

hepatitis C virus internal ribosome entry site RNA. *Proceedings of the National Academy of Sciences of the United States of America,* 101**,** 16990-16995.

JUBIN, R., VANTUNO, N. E., KIEFT, J. S., MURRAY, M. G., DOUDNA, J. A., LAU, J. Y. N. & BAROUDY, B. M. 2000. Hepatitis C virus internal ribosome entry site (IRES) stem loop IIId contains a phylogenetically conserved GGG triplet essential for translation and IRES folding. *Journal of Virology,* 74**,** 10430-10437.

KATO, N., HIJIKATA, M., OOTSUYAMA, Y., NAKAGAWA, M., OHKOSHI, S., SUGIMURA, T. & SHIMOTOHNO, K. 1990. Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. *Proc Natl Acad Sci U S A,* 87**,** 9524-8.

LOCKER, N., EASTON, L. E. & LUKAVSKY, P. J. 2007. HCV and CSFVIRES domain II mediate eIF2 release during 80S ribosome assembly. *Embo Journal,* 26**,** 795-805.

PESTOVA, T. V., SHATSKY, I. N., FLETCHER, S. P., JACKSON, R. J. & HELLEN, C. U. 1998. A prokaryotic-like mode of cytoplasmic eukaryotic ribosome binding to the initiation codon during internal translation initiation of hepatitis C and classical swine fever virus RNAs. *Genes Dev,* 12**,** 67-83.

PICKETT, B. E., GREER, D. S., ZHANG, Y., STEWART, L., ZHOU, L., SUN, G., GU, Z., KUMAR, S., ZAREMBA, S., LARSEN, C. N., JEN, W., KLEM, E. B. & SCHEUERMANN, R. H. 2012. Virus pathogen database and analysis resource (ViPR): a comprehensive bioinformatics database and analysis resource for the coronavirus research community. *Viruses,* 4**,** 3209-26.

QUADE, N., BOEHRINGER, D., LEIBUNDGUT, M., VAN DEN HEUVEL, J. & BAN, N. 2015. Cryo-EM structure of Hepatitis C virus IRES bound to the human ribosome at 3.9-A resolution. *Nat Commun,* 6**,** 7646.

SIZOVA, D. V., KOLUPAEVA, V. G., PESTOVA, T. V., SHATSKY, I. N. & HELLEN, C. U. 1998. Specific interaction of eukaryotic translation initiation factor 3 with the 5' nontranslated regions of hepatitis C virus and classical swine fever virus RNAs. *Journal of Virology,* 72**,** 4775-82.

TSUKIYAMAKOHARA, K., IIZUKA, N., KOHARA, M. & NOMOTO, A. 1992. Internal Ribosome Entry Site within Hepatitis-C Virus-RNA. *Journal of Virology,* 66**,** 1476-1483.

TULLER, T., WALDMAN, Y. Y., KUPIEC, M. & RUPPIN, E. 2010. Translation efficiency is determined by both codon bias and folding energy. *Proc Natl Acad Sci U S A,* 107**,** 3645-50.

VASSILAKI, N., FRIEBE, P., MEULEMAN, P., KALLIS, S., KAUL, A., PARANHOS-BACCALA, G., LEROUX-ROELS, G., MAVROMARA, P. & BARTENSCHLAGER, R. 2008. Role of the Hepatitis C Virus

Core+1 Open Reading Frame and Core cis-Acting RNA Elements in Viral RNA Translation and Replication. *Journal of Virology,* 82**,** 11503-11515.

YAMAMOTO, H., COLLIER, M., LOERKE, J., ISMER, J., SCHMIDT, A., HILAL, T., SPRINK, T., YAMAMOTO, K., MIELKE, T., BURGER, J., SHAIKH, T. R., DABROWSKI, M., HILDEBRAND, P. W., SCHEERER, P. & SPAHN, C. M. 2015. Molecular architecture of the ribosome-bound Hepatitis C Virus internal ribosomal entry site RNA. *Embo Journal,* 34**,** 3042-58.

YAMAMOTO, H., UNBEHAUN, A., LOERKE, J., BEHRMANN, E., COLLIER, M., BURGER, J., MIELKE, T. & SPAHN, C. M. 2014. Structure of the mammalian 80S initiation complex with initiation factor 5B on HCV-IRES RNA. *Nature Structural & Molecular Biology,* 21**,** 721-7.

Curriculum vitae

Anas Khawaja
Email: khawajaa@natur.cuni.cz
International mobility

Education

2007 – 2016 PhD in Molecular Biology, Charles University in Prague.
Supervisor: RNDr. Martin Pospíšek, Ph.D.

Research projects:

1. A study of the HCV IRES variability: An experimental approach coupled with design of a large-scale mutation database.
2. An interplay of codon bias and mRNA secondary structure acts as determinants of translation efficiency in HCV-core gene.

2006 – 2007 Master of Science (Molecular Biology), University of the Punjab.
Supervisor: Dr. Sajida Hassan

Research Project: Expression analysis of the Hepatitis C Virus core gene of Genotype 3a

2001 – 2005 Bachelor of Agriculture, University of Agriculture Faisalabad.

Internship: Surveying and identification of different vegetable viruses with the help of enzyme linked immuno sorbent assay (ELISA).

Scientific skills

Development of the HCV IRES variation database (HCVIVdb; *www.hcvivdb.org*).

Broad carnet of molecular biology skills (PCR, cloning, DGGE, TGGE, gene assembly).

Cell culture maintenance and protein biochemistry (Transfection, western blot, Immunoprecipitation).

Language: Fluency in English language.

Computing: Word, Excel, Powerpoint, Adobe photoshop, Adobe illustrator.

Selected Publications

Khawaja, A., Vopalensky, V. and Pospisek, M. (2014), Understanding the potential of hepatitis C virus internal ribosome entry site domains to modulate translation initiation via their structure and function. WIREs RNA. doi: 10.1002/wrna.1268

Floden, EW.*, **Khawaja, A.*,** Vopalensky, V. and Pospisek, M. (2016), HCVIVdb: The hepatitis-C IRES variation database. Manuscript submitted in BMC Microbiology. (**Authors contributed equally*).

Khawaja, A., Vopalensky, V., Roznovsky, L., Mrazek, J. and Pospisek, M. (2016), A study of the HCV IRES variability: An experimental approach coupled with design of a large-scale mutation database. Manuscript.

Conferences

ORAL Presentations

Khawaja, A., Vopalensky, V. and Pospisek, M. Inquiry into the variability of HCV IRES and its impact on function by developing and evaluation of a large-scale mutation database that also unfolds potential of some new nucleotides. RNA society 2013, Davos, Switzerland (June 2013).

Khawaja, A., Vopalensky, V. and Pospisek, M. Extensive investigation of the HCV IRES variability suggested new functions to some nucleotides. International RNA Club meeting, Prague, Czech Republic (Nov., 2011).

Khawaja, A., Vopalensky, V. and Pospisek, M. Extensive investigation of the HCV IRES variability suggested new functions to some nucleotides and revealed possible importance of domain II for the therapy. Society of General Microbiology (SGM), Harrogate International Centre, United Kingdom (April, 2011).

Khawaja, A., Vopalensky, V. and Pospisek, M. Analysis of HCV IRES Quasispecies. International RNA Club meeting, Prague, Czech Republic (Nov., 2008).

POSTER Presentations

Khawaja, A., Vopalensky, V. and Pospisek, M. Extensive investigation of the HCV IRES variability suggested new functions to some nucleotides. EMBO Conference. Series on Protein Synthesis and Translational Control. EMBL Heidelberg, Germany (September, 2011).

Khawaja, A., Vopalensky, V. and Pospisek, M. Extensive investigation of the HCV IRES variability suggested new functions to some nucleotides

and revealed possible importance of domains II and IIIb for the therapy. RNA Society 2011, Kyoto, Japan (June, 2011).

Khawaja, A., Vopalensky, V. and Pospisek, M. Point Mutation Determination of HCV-IRES Quasispecies Based on DGGE and TGGE Methods. International Symposium on Hepatitis C Virus and other related viruses, Nice, France (October, 2009).

Khawaja, A., Vopalensky, V. and Pospisek, M. Analysis of HCV- IRES Quasispecies. Federation of European Biochemical Socities (FEBS), Prague, Czech Republic (July, 2009).