Targeting the other genetic information coded by the viral RNA genomes

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**Diagram:**
- **5'UTR:** Replication (+) and Translation(+) and Translation (-)
- **3'UTR:** Replication (+) and Translation(+) and Translation (-)
- **IRES:** Translation initiation
- **PK1:** AUG initiation codon
- **PK2:** Translation (-)
- **5BSL3.1, 5BSL3.2, 5BSL3.3:** Replication (+) and Translation(+)
- **SL9074:** Replication (+)
- **5BSL3.1 ALT (9110):** Translation (-)
- **V:** Translation (-) and Replication (+)
- **3'X tail:** Dimerizable conformation
- **3'SLII:** Translation (-)
- **3'SLI:** Translation (-)
- **CRE:** Translation (--) and Replication (+) and Translation(+)
Abstract:
In addition to the protein coding information viral RNA genomes code functional information in structurally conserved units termed functional RNA domains. These RNA domains play essential roles in the viral cycle. Members of the *Flaviviridae* family are responsible of important worldwide human diseases (e.g. hepatitis C, dengue, zika, West Nile fever, among others). Their genome consists in a (+) single stranded RNA molecule, which contains numerous highly structurally conserved RNA domains. They represent a good model to study and characterize the functional roles of RNA domains in the regulation of essential viral processes (e.g. translation, replication). Understanding the molecular mechanisms behind their function is essential to understand the viral infective cycle. Interfering with the function of the genomic RNA domains offers a potential means of developing antiviral strategies. Nucleic acids tools and in particular aptamers are good candidates for targeting structural RNA domains. Besides its potential as therapeutics, aptamers also provide an excellent means for investigating the functionality of RNA domains in viral genomes.

Keywords: Viral RNA genomes; RNA structure/function; WNV; HCV; Aptamers
**Introduction:** RNA viruses have developed an information coding system that complements the protein coding one.

- This system uses discrete RNA units that fold into a specific structure for coding information that is essential for the completion of the viral cycle. These structural units are considered functional genomic RNA domains.
- These units can be grouped in complex folded RNA regions that are conserved among the viral isolates.
- More than the sequence the structure of the entire genome has to be preserved in order to achieve an efficient viral propagation. The structure of the RNA domains determines their function.
Introduction

**Hepatitis C virus (HCV)** belonging to the *Flaviviridae* family, *Hepacivirus* genus, is the etiologic agent of the hepatitis C, which affect the 3% of the world population.

The HCV genome is a ≈9,600 nt long, positive ssRNA molecule, which exhibits a highly variable sequence rate. It codes for a single ORF flanked by highly conserved untranslated regions (5’ and 3’ UTR). UTRs are reach in conserved structural units.
Introduction: HCV genome

Conserved structural domains are also distributed all throughout the protein-coding region (indicated by grey color bars)

Conserved domains represented by their predicted secondary structure.

The high structural conservation within a highly variable genome indicates that each structural unit plays an important function for the virus.
- The 5’ UTR and 3’ UTR are highly structured, both have been involved in viral replication and translation.
- A communication should exist between structural domains of both genomic ends to regulate the essential viral processes
Long-range RNA-RNA interaction between the essential domains IIId within the IRES at the 5’UTR and the 5BSL3.2 within the CRE at the 3’ end.
HCV RNA genome: Long-distance RNA-RNA interactions

The 5BSL3.2 negatively regulates the IRES activity.
Network of RNA-RNA interactions that govern the essential viral processes (replication, translation) and the switch between them. The 5BSL3.2 domain is at the core of this network that governs the progression of the HCV propagation.
Structure/function relationship of structural domains

A detailed structural analysis providing information at nucleotide level allowed us to conclude:

● The modulation of the activity of the 5’ genomic end (translation efficiency) by the 3’ end is achieved by promoting the conformational fine-tuning of the IRES essential domains III and IV.

● The 5’ genomic end promotes significant structural changes at the 3’ genomic end, mainly at the 3’X-tail region.

● This mutual structural influence may govern the regulation of the essential viral processes.

Nucleotides whose conformation is modified by the presence of the other genomic end are shown with red figures.
The 3’X-tail promotes the HCV genomic dimerization

The efficiency of genomic dimerization is controlled by structural elements outside the 3’X tail domain.

Confirms the existence of functional RNA-RNA interactions involving CRE-3’X tail and IRES-3’X tail. These interactions modulate the structure and determine the function of the 3’X tail.
Genomic structural RNA domains play essential roles in the regulation of the viral cycle.

Interfering with the function of these domains (impeding the interactions they are involved in, or modifying their structure) offers a potential means of treating HCV infection.

RNA molecules are excellent candidates to interfere with RNA structural domains.
Aptamers: oligonucleotides able to bind specifically and with high affinity to a target molecule

**Aptamer**

The term Aptamer comes from the Greek voice *haptein* - to bind to

Jack W Szostak

In *Nature* 346, 818-822 (30 August 1990) doi:10.1038/346818a0; Accepted

*In vitro* selection of RNA molecules that bind specific ligands

Andrew D. Ellington & Jack W. Szostak

Subpopulations of RNA molecules that bind specifically to a variety of organic dyes have been isolated from a population of random sequence RNA molecules. Roughly one in $10^{10}$ random sequence RNA molecules folds in such a way as to create a specific binding site for small ligands.

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Active molecule is Pegaptanib (a pegylated anti-VEGF RNA aptamer)

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SELEX

**Systematic Evolution of Ligands by Exponential Enrichment:**

Craig Tuerc; Larry Gold

*Science, New Series, Vol. 249, No. 4968 (Aug. 3, 1990), 505-510.*

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Aprobed by the FDA (Decembre 2004)
Aptamers selection scheme

SELEX: Systematic Evolution of Ligands by Exponential Enrichment

In vitro selection cycle
Aptamers targeting the HCV IRES

Inhibitory RNAs after six rounds of selection were classified in seven groups attending to the target site determined by sequence complementarity.

Most of the selected RNAs promoted significant inhibition of the IRES activity, up to 90%, in *in vitro* translation assays.
Anti-HCV activity of Aptamers targeting the HCV IRES in cell culture

Inhibition of HCV Translation (IRES activity) in Huh-7 cells

Inhibition of HCV replication in Huh-7 subgenomic replicon system
Aptamers targeting the HCV CRE

Anti HCV CRE RNA aptamers inhibit the viral replication up to 90% in a cellular subgenomic replication system.
Aptamers targeting the HCV CRE

RNA Aptamers targeting the essential CRE 5BSL3.2 domain compete the recruitment of the viral RNA polimerase (NS5B)
Aptamers targeting the HIV 5’ UTR

Structural analysis of isolated aptamers revealed a highly conserved 16 nt long consensus structural RNA domain. An *in silico* designed minimal RNA aptamer consists in a 4 bp helical region closed by an 8 nt long closing loop. Nucleotide sequence of the loop is complementary to the HIV-1 PolyA domain.
Anti HIV-1 5’UTR Aptamers

The RNA16(+) inhibits up to 80% HIV-1 viral particles production in a cell culture assay.

The RNA16(+) is the smaller aptamer molecule ever described.
Conclusions

- Viral RNA genomes have developed a storing information system that complements the protein coding one.
- This system codes essential information for the viral cycle.
- The information is coded in defined structural units. This units can be grouped in complex folded RNA regions that are highly conserved among the viral isolates. These structural domains play essential functions in the progression of infection.
- The functional RNA domains establish a dynamic complex network of RNA-RNA interactions and recruit specific cellular and/or viral factors for their functioning.
- In the case of HCV the CRE domain seems to play a central role in the regulation of the RNA-RNA interactions network, regulating the viral processes.
- Interfering with the activity (structure/function) of the functional genomic RNA domains offers a potential means of treating viral infections.
- RNA Aptamers targeting specific functional RNA domains are efficient antiviral agents.
Acknowledgments