Regulating mRNA translation with a kiss.

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ABSTRACT

Loop-loop interactions mediate the recognition between RNA hairpins leading to the formation of so-called kissing complexes. Both the size and the sequence of the loop are critical for ensuring stable interaction. Using in vitro selection we have characterized a few loop sequences that lead to the formation of highly stable kissing complexes. These sequences constitute targets of interest for the rational design of RNA stem loop ligands. Such an appropriate target sequence was identified in a sub-domain of the Internal Ribosomal Entry Site (IRES) of the Hepatitis C Virus (HCV) mRNA. We synthesized chemically-modified RNA hairpins and demonstrated that they specifically reduced the expression of a HCV IRES driven reporter gene in cultured cells.

RESULTS AND DISCUSSION

We prepared a library of RNA hairpins with a fixed stem and a randomized loop sequence. In order to cover the space of odd and even number of nucleotide residues in the loop we actually mixed two sub-libraries with 10 and 11 random positions. SELEX was carried out in a 20\text{mM} HEPES buffer pH 7.4 containing 20 \text{mM} sodium acetate, 140 \text{mM} potassium acetate and 3\text{mM} magnesium acetate. After incubation at room temperature the mixture was run on a native polyacrylamide gel. Retarded bands compared to individual RNA hairpins likely corresponded to RNA-RNA complexes. The material contained in these bands of reduced mobility was recovered and used for the next round of selection. After 4 rounds the candidates were cloned and sequenced. The comparison and alignment of selected sequences allowed the identification of a series of hairpins with partly complementary loops which were susceptible to form kissing complexes.

The loop of these selected hairpins were generally G,C rich and displayed up to 7 contiguous complementary nucleotides in the loops. In addition particular sequences were found in the loop of several selected hairpins. For instance candidates containing CCNY or its complement RNGG represented about 40% of the selected population.

We synthesized representatives of such hairpins and demonstrated by band-shift assay, UV-monitored thermal denaturation and surface plasmon resonance measurements that they did form complexes with a K_d of a few nM under the selection conditions. Moreover this complex was recognized by the E. coli protein Rop that is specific for kissing complexes, suggesting that hairpin aptamers did interact through the formation of a loop-loop helix.

The domain IV of the HCV IRES harbours the RNGG motif in its loop (7). Thus we designed a 2'-O-methyl hairpin aptamer (08041) with the CCNY sequence in the loop. We investigated the effect of this aptamer using a bicistronic luciferase construct pIRF in which the \textit{Renilla} luciferase reading frame translation is under the control of the HCV IRES (8). The pIRF RNA/aptamer complex was transfected into Huh-7 human hepatocarcinoma cells and luciferase activity was evaluated. This aptamer inhibits the translation of the \textit{Renilla} gene in a dose dependent manner with an IC\textsubscript{50} value of 800nM (figure 1). No significant inhibition of the Firefly luciferase was observed in the presence of our aptamer. The inhibition is highly specific of the loop sequence. Indeed no effect was observed with a
control hairpin (08017) displaying a single mutated residue in the loop.

To further assess the specificity of this inhibitory effect we transfected the same reporter bicistronic construct in which the HCV IRES was replaced by the encephalomyocarditis virus (EMCV) IRES. No significant effect was observed in the presence of 1μM of the aptamer 08041 (not shown).

![Graph showing effect of hairpin aptamer on luciferase expression](https://academic.oup.com/nass/article-abstract/52/1/711/1109005)

**Figure 1.** Effect of the hairpin aptamer on the translation of Luciferase bicistronic transcript. The pT7RF RNA/aptamer complex was transfected into Huh-7 cells. The luciferase expression (Rluc/Ftuc) is given relative to a sample without aptamer. Grey bar, aptamer 08041; black bar, control hairpin 08017.

**CONCLUSION**

RNA hairpins with a G,C rich loop are susceptible to generate kissing complexes of high stability. The tetranucleotide sequence CCNY is prone to the formation of highly stable loop-loop interaction. A 2'-O-methyl-RNA hairpin targeted to the loop of the domain IV of the HCV IRES reduces the translation of a downstream gene by more than 60% in cultured Huh-7 cells. Therefore kissing aptamers extends the repertoire of oligonucleotides of interest for the regulation of gene expression and of potential therapeutic value.

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