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Characterization of RNA aptamer binding to Rift Valley fever virus nucleocapsid protein

Ilona C. Csik
The University Of Montana, ilona.csik@umontana.edu

Katherine Hornak
The University Of Montana, katherine.hornak@umontana.edu

Characterization of RNA Aptamer Binding to Rift Valley Fever Virus Nucleocapsid Protein

Ilona Csik and Katherine Hornak

Division of Biological Sciences, The University of Montana, Missoula, Montana 59812

Background

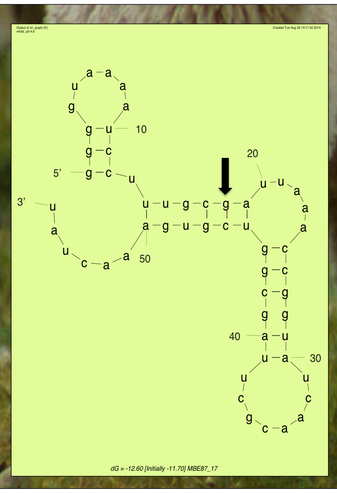
Rift Valley fever virus (RVFV) is a mosquito-borne virus that can cause hemorrhagic fever in humans and miscarriage in livestock. Originally endemic to sub-Saharan Africa, RVFV has now spread to the Arabian Peninsula as well.[1] There is currently no cure for RVFV. Viral nucleocapsid protein (N) binds to viral RNA during RVFV replication and transcription, and it coats the viral genome to protect the viral genetic material.[2, 3] Thus, disruption of N-RNA interaction is a good potential therapeutic target for a new class of antiviral drugs. To exploit this target, it is essential to understand how N recognizes viral RNA sequences specifically. The Lodmell laboratory previously discovered a small RNA shown to bind N with high affinity called MBE87.[4] MBE87 contains two GAUU nucleotide motifs that we hypothesize to be important for N binding. To test this hypothesis we designed and constructed RNAs with the same predicted secondary structure as MBE87 but with GAUU motifs at different positions in the structure, and assessed the binding affinity of N to the different RNA constructs by electrophoretic mobility shift assays.

Research Question

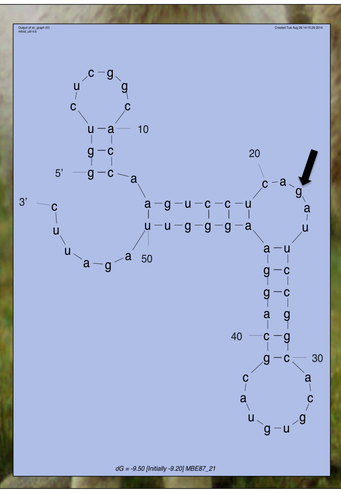
Does Rift Valley fever virus nucleocapsid protein recognize a primary nucleotide sequence, a specific secondary structure, or both when binding RNA?

Methods and Materials

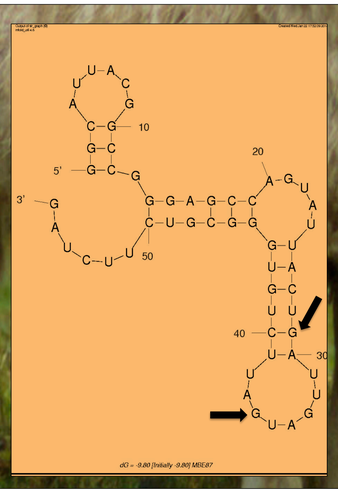
In-silico designed aptamer sequences with identical predicted secondary structures but different primary sequences were cloned into expression plasmids and amplified in *E. coli* cells. Large-scale DNA preparations of these plasmids were preformed and used as templates for *in vitro* RNA transcriptions to make the novel RNA aptamers with strategically placed GAUU motifs. α-^{32}P UTP was used in the transcription reaction to produce internally (^{32}P) labeled RNA. Using the MBE87 aptamer and an aptamer devoid of GAUU motifs as controls, electrophoretic mobility shift assays were performed to qualitatively compare the extent of RNA-N binding between constructs. N protein concentration started at 10 μM and was diluted 1.5-fold over 13 reactions. 100 μM suramin (in DMSO) was present in reactions. Controls – RNA alone and N-RNA without suramin – were each prepared with 1 μL of DMSO present to control for the suramin stock solvent. 11 800 counts of labeled RNA (2 μL) were then added to reactions. Reactions were incubated at room temperature for 30 minutes. Reactions and controls were run on 6% PA-1x TB gels. Percent of RNA bound to N was quantified using ImageGauge software (Fuji Medical Systems).



dG = -12.60 [Initially -11.70] MBE87_17

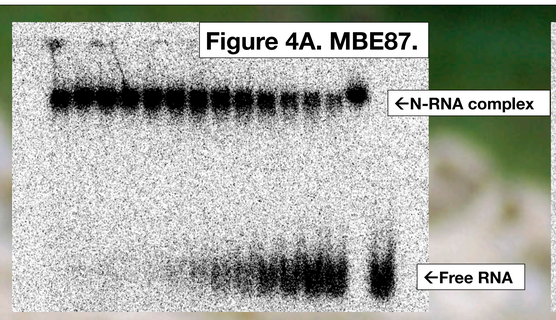


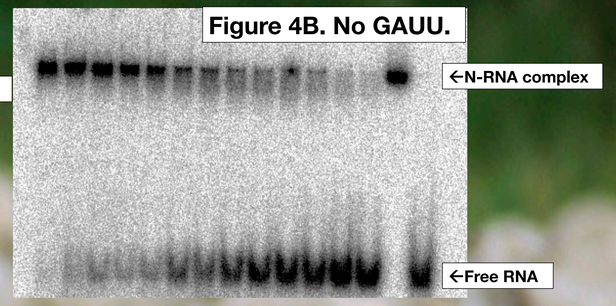
dG = -9.50 [Initially -9.20] MBE87_21

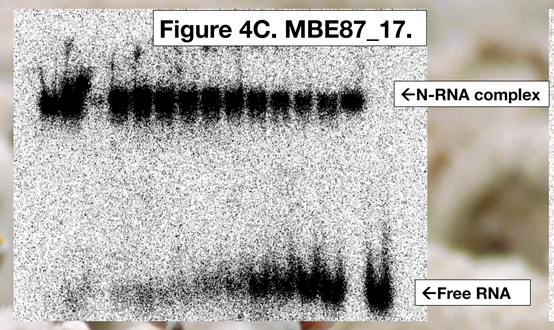


dG = -9.80 [Initially -9.80] MBE87

Figures 1-3. From left to right: Figure 1 shows the aptamer MBE87_17. Figure 2 shows the aptamer MBE87_21. 17 and 21 signify GAUU placement in the aptamers. Figure 3 shows the aptamer MBE87, the high affinity binder from which the other constructs were derived using a genetic algorithm created by Dr. Douglas Raiford (UM). The algorithm allows for relocation of the GAUU motif while retaining the secondary structure of MBE87.







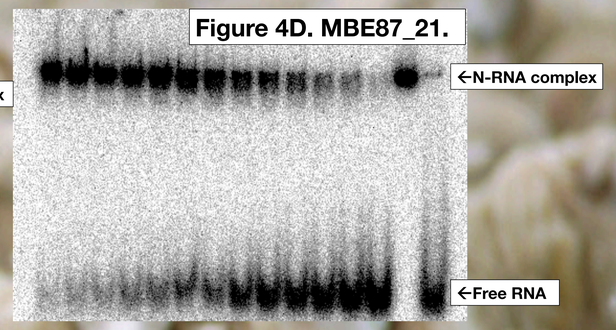


Figure 4. Electrophoretic mobility shift results for constructs. N protein concentration starts at 10 μM in the left-most lane and is diluted 1.5-fold (6.7 μM, 4.4 μM, 2.96 μM, 1.98 μM, 1.32 μM, 0.88 μM, 0.59 μM, 0.39 μM, 0.26 μM, 0.17 μM, 0.12 μM, 0.077 μM from left to right – lanes 2-13). The N-RNA control is in lane 14; the RNA control is in lane 15 (right-most lane) for all constructs. At higher N concentration, more RNA is expected to be bound and thus present in the top band.

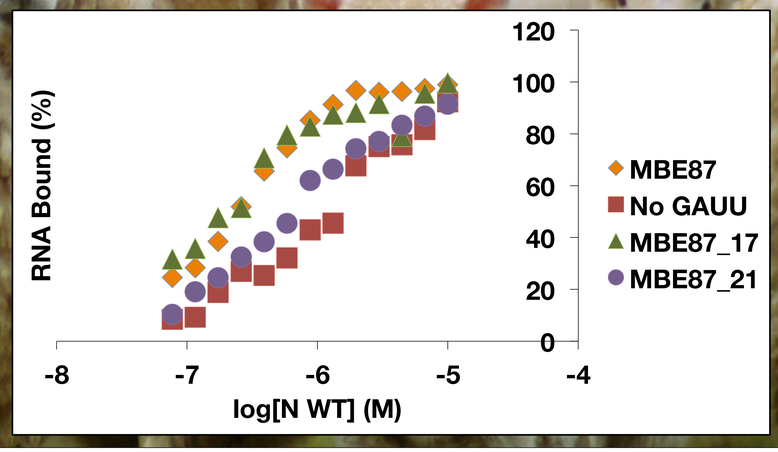


Figure 5. EMSA band intensity data from Figure 4 for all four constructs was quantified using ImageGauge software (Fuji). These binding data indicate higher binding affinity of the MBE87 and MBE87_17 constructs.

Conclusions

We determined that MBE87 binds to N with the highest affinity. MBE87_17 also binds to N with high affinity. MBE87_21 binds to N with less affinity than MBE87_17. The construct without the GAUU motif binds to N with lower affinity as well, indicating that the presence of the GAUU motif may be important for RNA recognition by N. However, these results suggest that the positioning of the GAUU in relation to secondary structural elements may be important for recognition and binding by N, too. MBE87 and MBE87_17, with GAUU positioned partially in a double-stranded region, bound to N with higher affinity than MBE87_21, with GAUU positioned only in a largely single-stranded region. It is possible that both GAUUs situated in each of these 2° elements are needed for optimal binding, as MBE87 still binds with slightly higher affinity than MBE87_17. It is also possible that N recognizes a sequence motif other than GAUU entirely, or that N simply requires a recognizable general 2° structure to bind aptamers. Studies to investigate further possibilities are underway.

Citations

[1]Balkhy, HH, Memish, ZA. 2003. Rift Valley fever: an uninvited zoonosis in the Arabian peninsula. *Int. J. Antimicrob.l Agents*. 21:153–157.
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[4]Ellenbecker, M, Sears, L, Li, P, Lanchy, JM, Lodmell, JS. 2012. Characterization of RNA aptamers directed against the nucleocapsid protein of Rift Valley fever virus. *Antiviral Research*. 93:330-339.
Background photo: Mula Eshet, Robert Harding World Imagery, Corbis

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