Candidate RNAs for Domain 3 of the Foot-and-Mouth-Disease Virus Internal Ribosome Entry Site

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Introduction

The foot-and-mouth-disease virus (FMDV) utilizes non-canonical translation initiation for viral protein synthesis, by forming a specific RNA structure called internal ribosome entry site (IRES). Domain 3 in FMDV IRES is phylogenetically conserved and highly structured; it contains four-way junctions where intramolecular RNA–RNA interactions serve as a scaffold for the RNA to fold for efficient IRES activity. Although the 3D structure of domain 3 is crucial to exploring and deciphering the initiation mechanism of translation, little is known. Here, we employ a combination of various modeling approaches to propose candidate tertiary structures for the apical region of domain 3, thought to be crucial for IRES function. We begin by modeling junction topology candidates and build atomic 3D models consistent with available experimental data. We then investigate each of the four candidate 3D structures by molecular dynamics simulations to determine the most energetically favorable configurations and to analyze specific tertiary interactions.

RNA target structure

Domain 3 of FMDV IRES is a self-folding RNA that is 214 nt long. We consider the sequence of the FMDV C-S8 IRES.

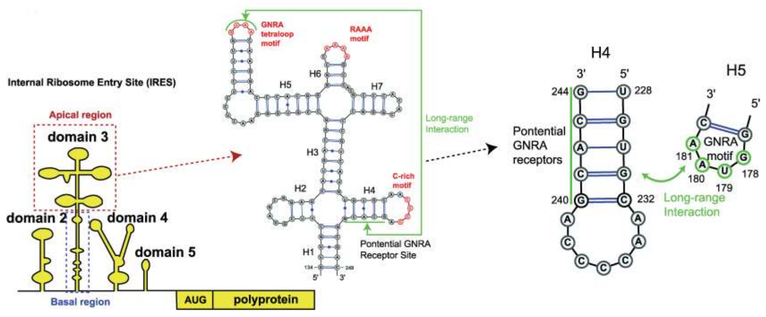

Figure 1. Global organization of FMDV IRES and secondary structure of the target domain 3

Computational procedure for modeling multiple four-way RNA junction structures

To tackle multiple consecutive four-way RNA junctions, we use a divide-and-conquer approach by partitioning the large complex. Each four-way junction is analyzed with regards to the loop size of single strands between helices; this analysis is coupled to the Junction-Explorer program[1] to help determine coaxial stacking patterns and helical arrangements. Using the predicted topology for each four-way junction, we search for all possible combinations of the multiple four-way junctions to produce combined structures. These potential topologies for the secondary structures are then refined further by incorporating experimental data as constraints. Using state-of-the-art 3D modeling programs[2], we build RNA 3D models of FMDV IRES domain 3 combined with experimental data. The four resulting candidate 3D models correspond to the junction topology models.

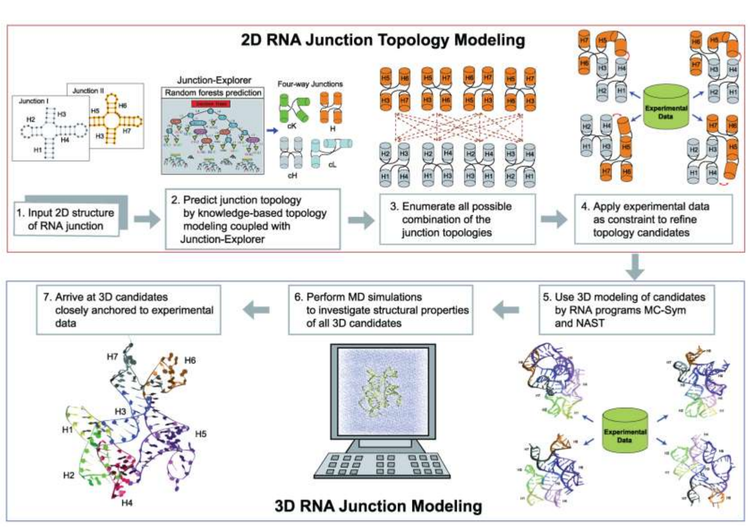

Figure 2. Modeling multiple four-way RNA junctions

Assessment of structural properties using MD

We use MD to explore the feasibility of our structural candidates; specifically to investigate structure stability and potential long-range interactions suggested by experimental data. The trajectory for Model C shows that the two adenosines retain a distance <3 Å. In contrast, only the first adenosine A_{180} of Models A, B and D retain a distance <4 Å during the initial 12, 15 and 26 ns, respectively. In Model C, the average distance between C_{232}/G_{240} pair and A_{180} is 2.1 ± 0.59 Å while C_{231}/G_{241} pair and A_{181} is 2.0 ± 0.20 Å. These findings suggest that the C_{232}/G_{240} and C_{231}/G_{241} pairs may be the target receptors of A_{180} and A_{181} residues, respectively.

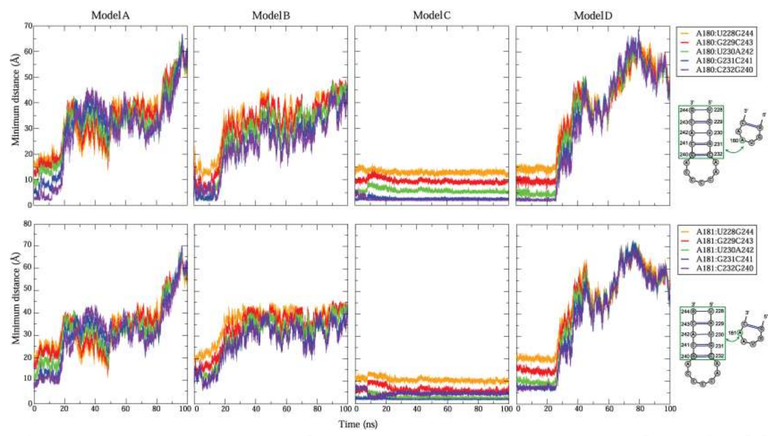

Figure 3. RNA–RNA long-range interactions identified by distance measures of atoms between two adenosine—A_{180} and A_{181}—in GUAA tetraloop and its potential receptors during the MD trajectories.

Analysis of tertiary interactions in Model C

Three long-range interactions occur sequentially: at ~7, 20 and 22 ns, involving A_{180}, A_{181} and U_{179}, respectively. These cooperative long-range interactions help stabilize the IRES domain 3.

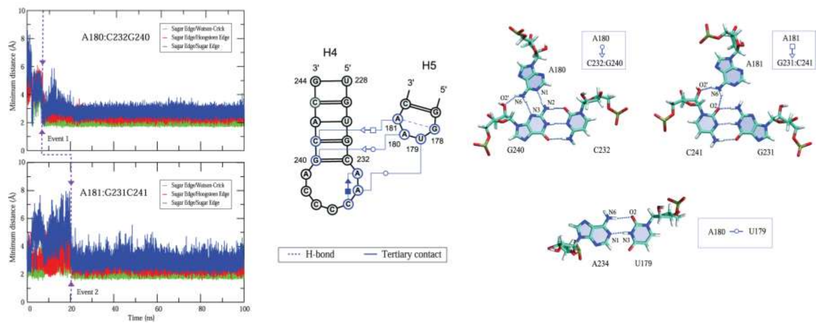

Figure 4. Intramolecular RNA–RNA long-range interactions involving GUAA hairpin loop during the Model C MD simulation.

The role of tertiary contacts for structural organization

The tertiary contacts in IRES domain 3 may restrict these fluctuations and therefore help recruit ribosomes for viral protein synthesis.

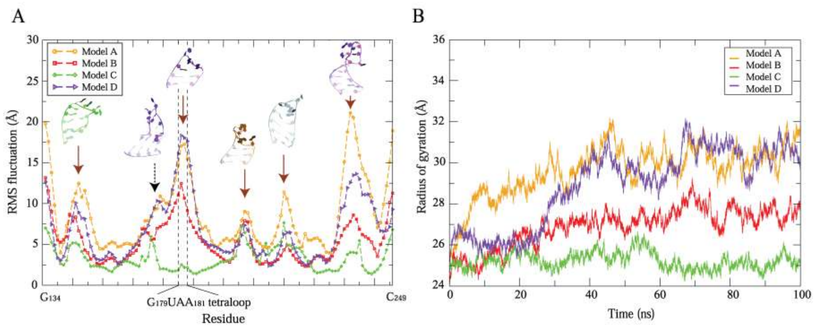

Figure 5. RMS fluctuations (**A**) and Rg (**B**) measures for four candidate 3D models. In the RMS fluctuations, high peaks (dotted black arrow for internal loop and solid brown arrows for hairpins) correspond to unpaired regions shown as solid color in the structures.

Proposed 3D structure for the apical region in domain 3

Time-averaged tertiary structure of domain 3 taken from the 100 ns dynamics data (top middle), where the long-range interactions occur between helices $H_4$and H_5 (details shown at bottom right). Both Junctions I and II contain two coaxial stacking, parallel to each other and Junction I with a crossing in the single-stranded region (bottom-left and -middle for Junctions I and II, respectively). Both junctions are planar locally and are arranged in a perpendicular orientation to each other globally (top right); note that the three helices H_4, H_3 and H_7 are coaxially stacked all together.

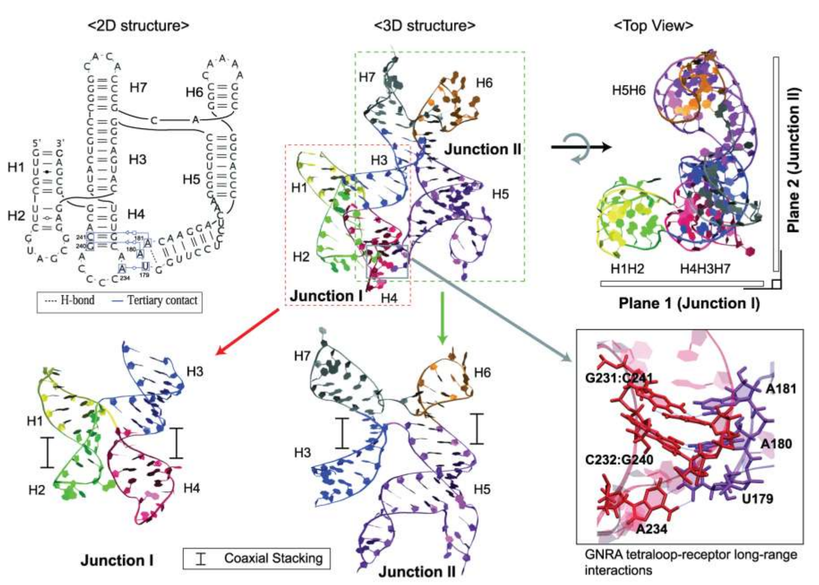

Figure 6. Time-averaged tertiary structure of apical region in domain 3

Domain 3 consists of basal and apical regions; the corresponding structural elements are an internal loop and four-way junctions, respectively. The minimum distance between the two regions is 23.5 Å that the basal region is not likely involved in RNA

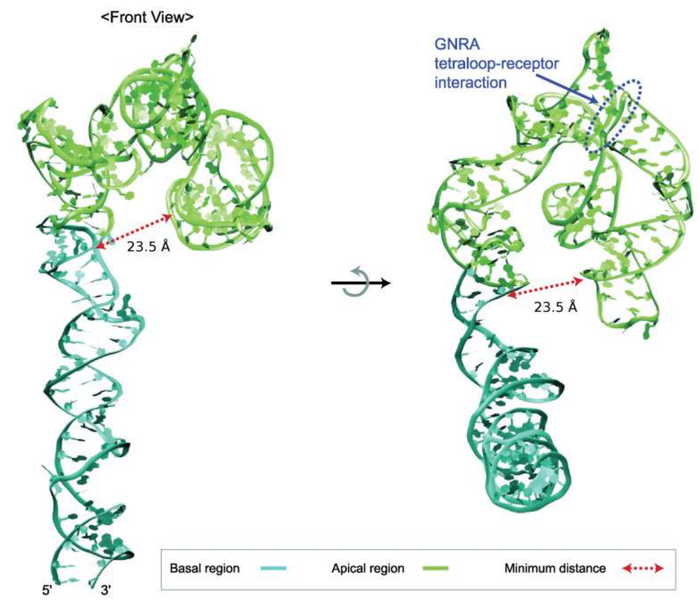

Figure 7. 3D model of the entire sequence in domain 3

Conclusions

We propose a theoretically feasible tertiary structure for the apical region in FMDV IRES domain 3; the overall 3D configuration and the suggested long-range interactions in the domain 3 provide insights into the potential role of the long-range interactions for structural stability and organization[3].

Acknowledgements

This work was supported by National Science Foundation [EMT award CCF-0727001 to T.S.]; and National Institutes of Health [grants GM081410 and GM100469 to T.S.]; and a MacCracken Fellowship to S.J.

1 Laing et al. *Predicting coaxial helical stacking in RNA junctions*. Nucleic Acids Res. 40(2):487-98 (2012)
2 Parisien et al. The MC-Fold and MC-Sym pipeline infers RNA structure from sequence data. Nature 452(7183):51-5 (2008)
3 Jung and Schlick. Candidate RNA structures for domain 3 of the foot-and-mouth-disease virus internal ribosome entry site. Nucleic Acids Res. (2012)