Determining the Location of Nucleotides in RNA Using Hydroxyl Radicals

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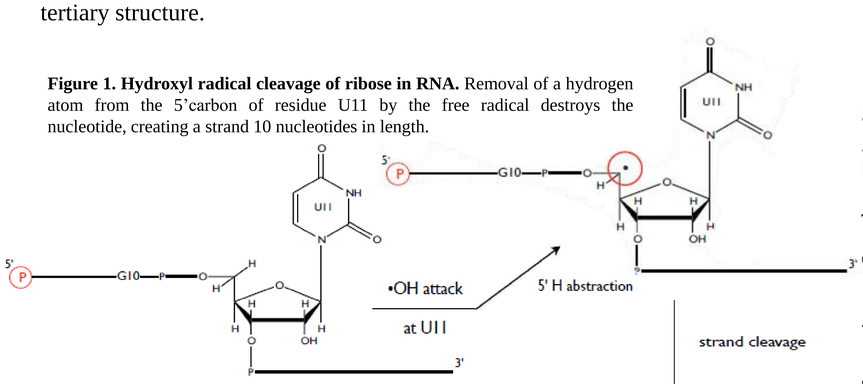
Abstract

Although RNA contributes to every aspect of a cell's function, the nucleotide sequence is where exact structure ends for most RNA'. Molecular shape commonly determines biological function, so a technique to determine an RNA's overall or tertiary structure is crucial to understand its function. We are creating and verifying a new technique to determine if specific nucleotides are on the inside or outside of an RNA molecule, both in vitro and in living cells, using the hydroxyl radical (an OH molecule with an unpaired electron). The tertiary structure of RNA can then be modeled using its secondary structure and the information from the hydroxyl radical experiment. The hydroxyl radical removes a hydrogen atom from a ribose in the RNA backbone and causes the strand to break. When a ribose is on the outside of the folded RNA, there is a higher chance that a backbone hydrogen is removed. Therefore, more breaks occur at nucleotides on the outside of folded RNA. The RNA fragments are sequenced to determine the amount of cleaved product at each nucleotide. The intensity of a particular RNA fragment is then a representation of how accessible an individual nucleotide is to the radical. Results are plotted and quantified using an in-lab program, RobFinder, and QuShape, from the University of North Carolina at Chapel Hill. By conducting the reaction using the M-Box riboswitch, an RNA with known structure, we can compare our results to the literature to determine if the method is viable. While more known RNA structures will need to be tested for full confirmation, our results suggest that this hydroxyl radical method for determining the location of nucleotides in a folded RNA gives results consistent with the literature.

Background

With the advent of hydroxyl radical probing we can visualize the structure of an an RNA molecule at a single nucleotide level. Busan and Weeks suggest that computation can be used to predict the tertiary structure of RNA, with two experimental inputs: the RNA secondary structure and hydroxyl radical cleavage data [1]. Having to accommodate both electrostatic interactions and nucleotide location on the inside or outside of the folded RNA helps a program simulate tertiary structure.

Figure 1. Hydroxyl radical cleavage of ribose in RNA. Removal of a hydrogen atom from the 5'carbon of residue U11 by the free radical destroys the nucleotide, creating a strand 10 nucleotides in length.



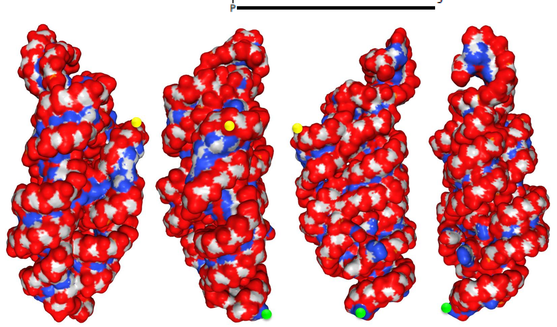


Figure 2. X-Ray crystal structure of the M-Box Riboswitch, with successive 90° rotations. Yellow and green dots are randomly chosen nucleotides to give perspective.

Methods

The M-Box RNA molecule was maintained at 37 °C for 20 minutes. Iron(II) EDTA, hydrogen peroxide, and sodium ascorbate were then added to produce hydroxyl radicals to introduce strand breaks. Reverse transcriptase was then used to produce cDNA fragments from the cleaved RNA fragments. The cDNA fragments were run through a Capillary Electrophoresis Sequencer (CEQ) to determine cleavage frequency at each nucleotide.

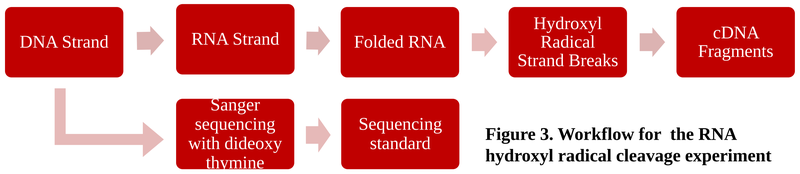


Figure 3. Workflow for the RNA hydroxyl radical cleavage experiment

Results

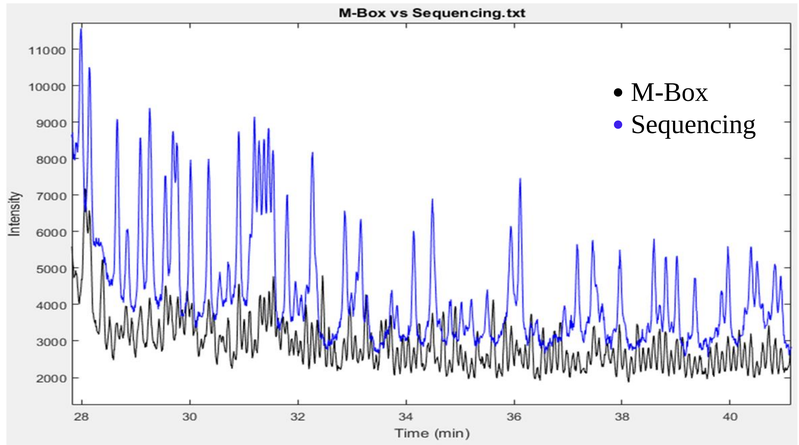


Figure 4. Raw capillary electrophoresis data plotted using RobFinder. The sequencing pattern (blue) allows for determination of the identities of nucleotides cleaved by hydroxyl radical (black).

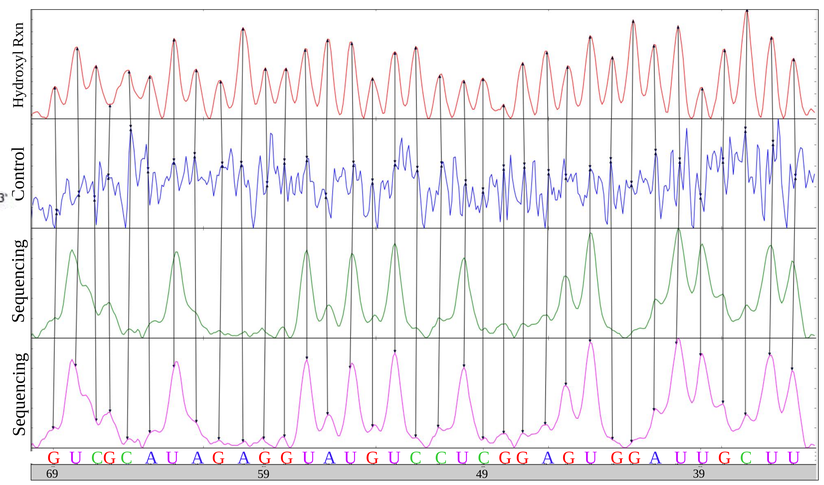


Figure 5. Analysis of data using QuShape. Data from the M-Box RNA cleavage reaction (nucleotides 35-69) are lined up with sequencing data to determine which peak corresponds to which nucleotide. The taller peaks in the sequencing patterns correspond to RNA uracil nucleotides due to the dideoxythymine used in the DNA sequencing reaction.

Conclusion

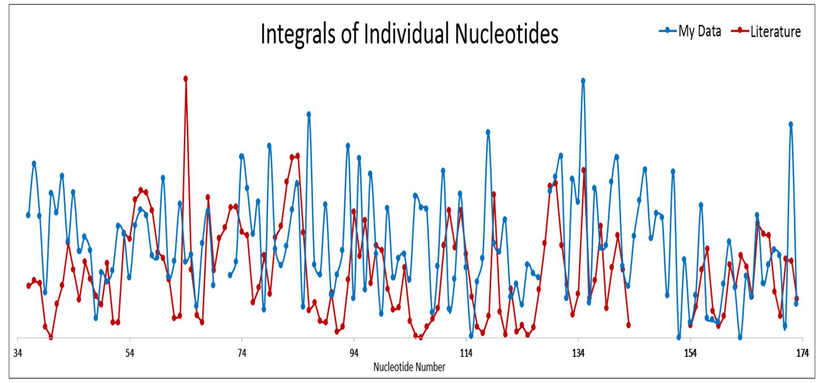


Figure 6. Comparison of integrals of nucleotides 35-172, computed by QuShape, for my data and the literature [2]. The overall shape of the cleavage patterns are similar, but there are some discrepancies.

We have found that the hydroxyl radical experiment produces similar results to the available literature. While there are some minor discrepancies, other experiments in the lab have produced results even closer to the literature. This is promising and suggests that the next steps in the project can be taken.

Future Work

The Tullius Lab at Boston University will continue to verify the hydroxyl radical reaction with known RNA structures. One of the next steps, in conjunction with Dr. Aaron Beeler's lab at Boston University, will be to run the cleavage reaction using flow chemistry, a technique that requires little to no human involvement. This will take human error out of the experiment. Another future step will be the creation of an RNA-seq library from the hydroxyl radical reaction products. Once the new method has been verified, the lab plans to map the structures of all the mRNA in a transcriptome in a single experiment.

References

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Acknowledgments

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