Secondary Structure Analysis of Catalytic RNAs

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Abstract

There is strong scientific evidence that in early forms of life RNA fulfilled the hereditary and catalytic roles corresponding to DNA and proteins in today's life forms. Catalytic RNAs are called ribozymes.

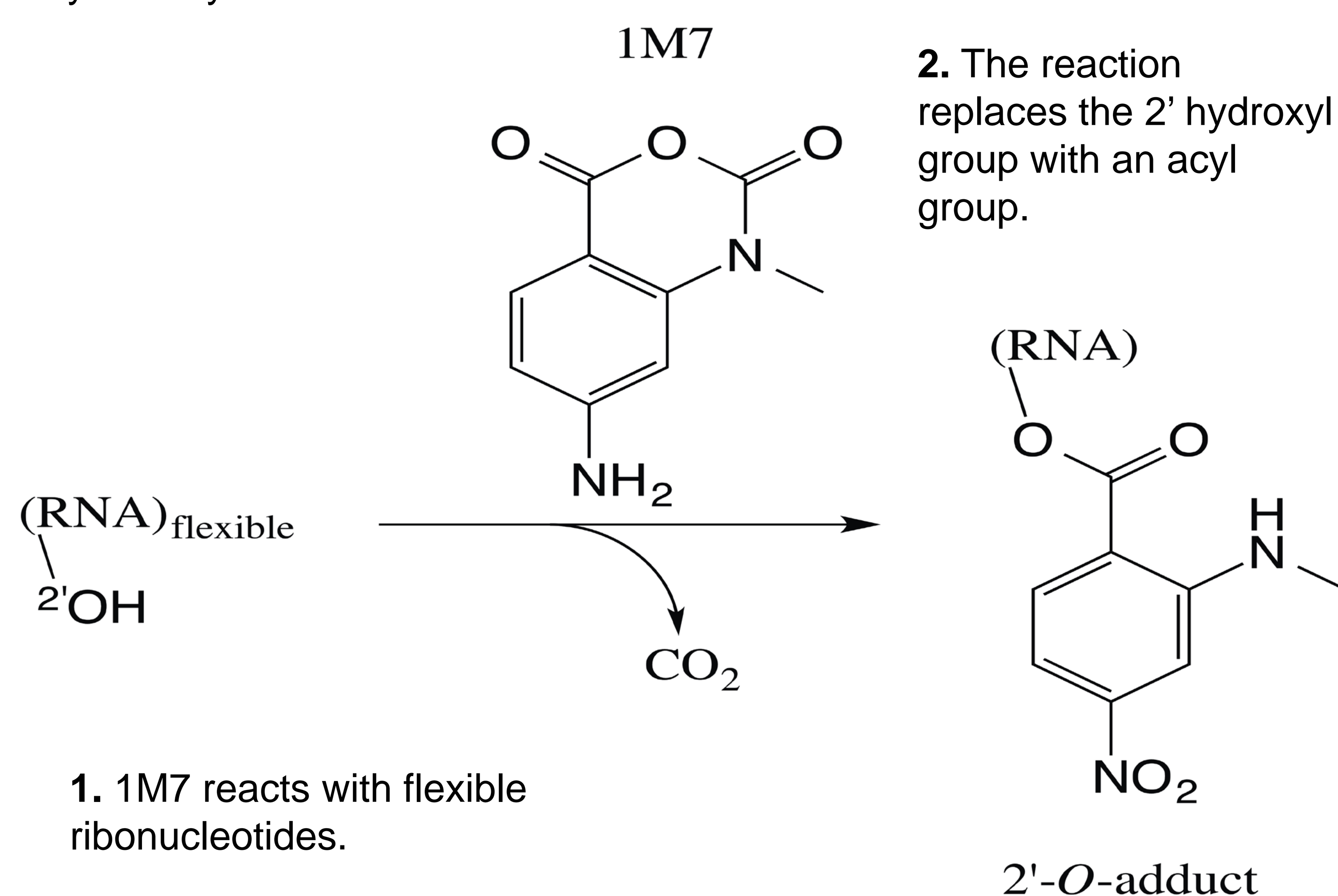
RNA secondary structure formation is essential for RNA catalysis. A ribozyme's secondary structure forms when some ribonucleotides base pair with other ribonucleotides. Unpaired ribonucleotides are flexible and are readily altered by specific chemicals.

In this investigation, the secondary structure of R5_5C1, a triphosphorylation ribozyme discovered by the Müller lab[1], was analyzed using selective 2'-hydroxyl acylation and primer extension (SHAPE)[2]. The Mfold RNA folding program was used to construct a possible secondary structure based on the SHAPE results[3].

Triphosphorylation ribozymes attach a triphosphate group to a ribonucleotide, using trimetaphosphate (Tmp). Tmp is a prebiotically plausible molecule because it can be generated from phosphites[4], which appear to have existed at 0.1 mM concentration in parts of the prebiotic ocean[5]. The Müller lab is interested in triphosphorylation ribozymes because they could provide an RNA world organism with chemical energy.

Selective Acylation

During summer research, various DNA templates that coded for ribozyme clone R5_5C1 were amplified via PCR and transcribed in vitro. The resulting RNA was purified by denaturing polyacrylamide gel electrophoresis (PAGE). The RNA was then selectively acylated by 1-methyl-7-nitroisatoic anhydride (1M7). Only flexible nucleotides are acylated by 1M7.

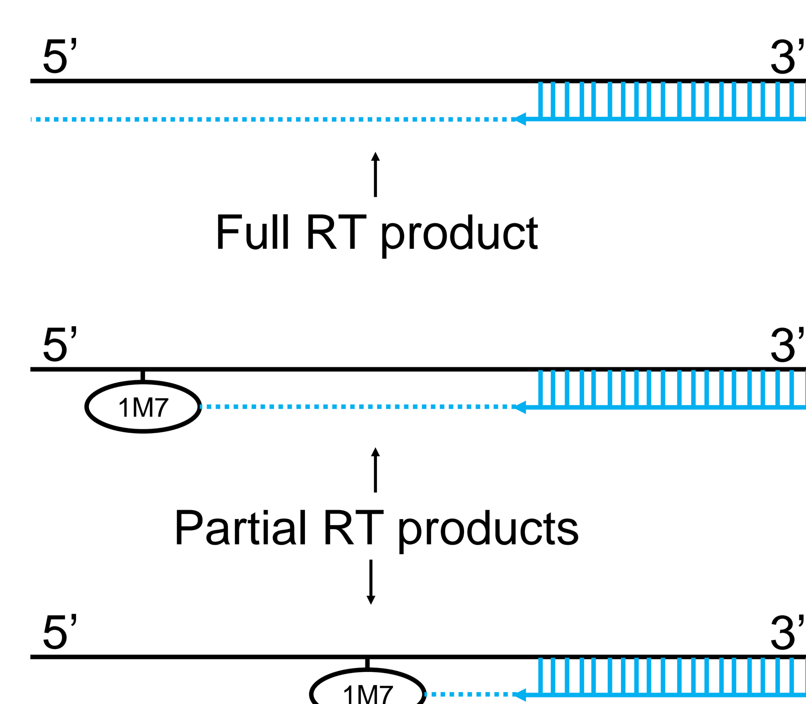


1. 1M7 reacts with flexible ribonucleotides.

2. The reaction replaces the 2' hydroxyl group with an acyl group.

Primer Extension

The amount of 1M7 in the selective acylation reaction was kept low to avoid more than one acylation per ribozyme. The ribozymes were then reverse transcribed with a primer that carried a 5'-[^{32}P] radiolabel. The reverse transcription (RT) of the ribozymes began at a 21-nucleotide primer sequence and continued towards the 5' terminus.



1. Reverse transcription terminated at each acylated nucleotide, since reverse transcriptase cannot pass acylated nucleotides.

2. The lengths of the formed DNA strands correspond to the position of each acylated nucleotide.

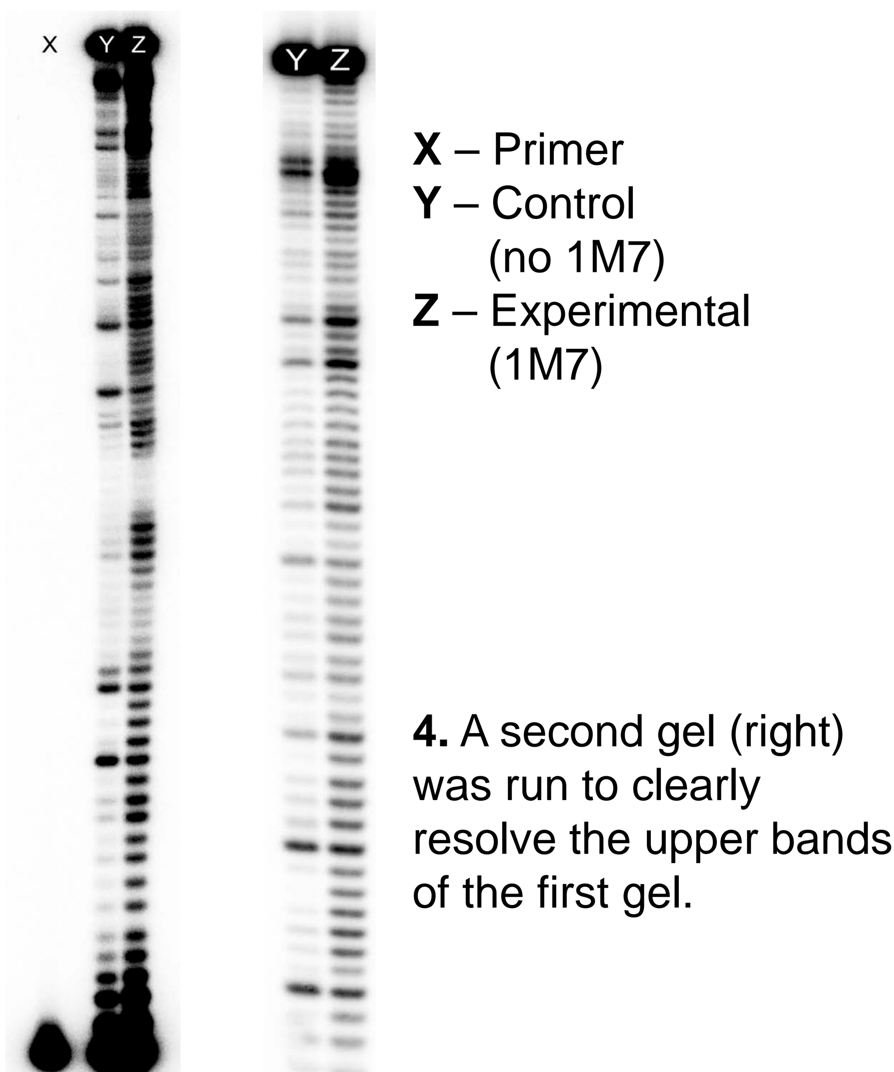
Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) is able to determine the length of each DNA fragment with single-nucleotide resolution. The gel image below was generated by a phosphoimager, which visualizes the β-radiation emitted onto a screen by the radiolabeled primer.

1. The length of each band of DNA fragments can be determined with a resolution of a single nucleotide.

2. The intensity of each band indicates the fraction of RT products terminated at the band's position. Dark bands only present in the 1M7 lane show that the nucleotide at the next position reacted with 1M7.

3. The length corresponding to each band is used to determine the position of each acylated nucleotide.

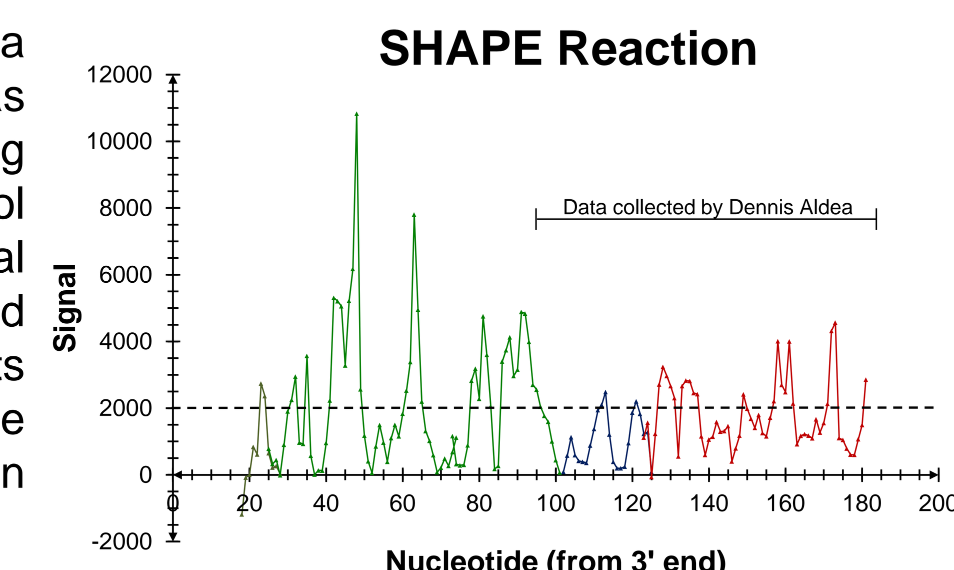


X – Primer
Y – Control (no 1M7)
Z – Experimental (1M7)

4. A second gel (right) was run to clearly resolve the upper bands of the first gel.

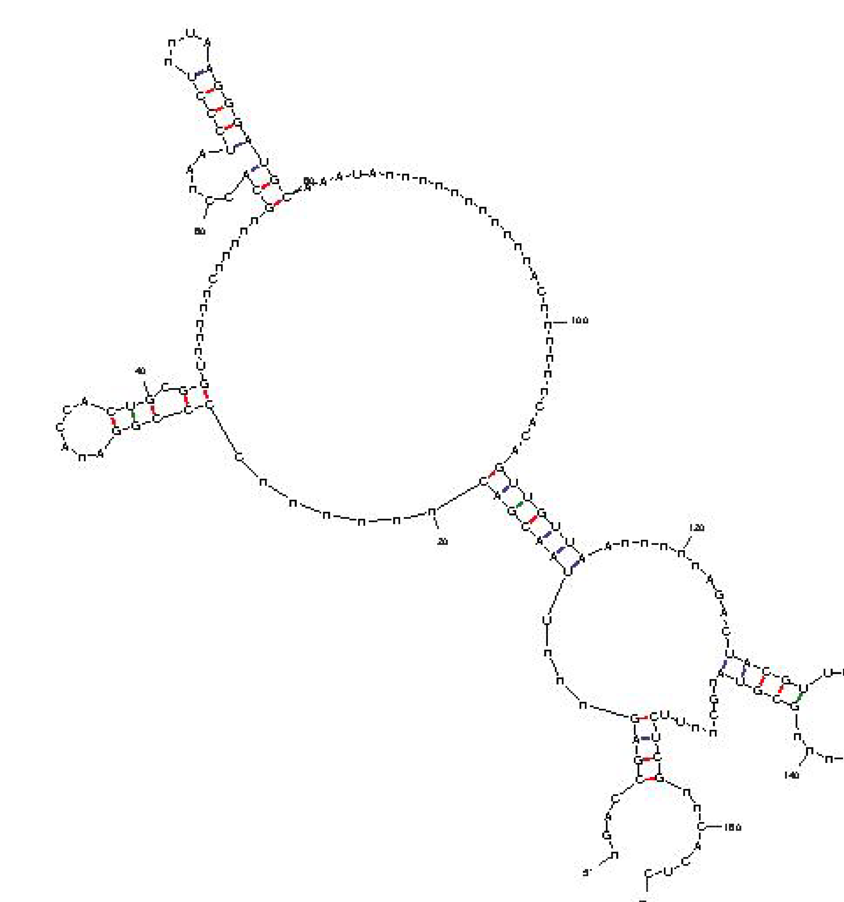
Results

For each nucleotide, a signal strength was calculated by subtracting the intensity of its control band from its experimental band. The values obtained from multiple experiments were scaled to form the continuous series shown above.



For each nucleotide, a signal strength was calculated by subtracting the intensity of its control band from its experimental band. The values obtained from multiple experiments were scaled to form the continuous series shown above.

Nucleotides with low signals are not very reactive with 1M7, and are likely paired with other nucleotides. In contrast, nucleotides with high signals are likely unpaired.



The secondary structure diagram was constructed by Zuker's Mfold software based on the SHAPE results. Nucleotides with signals greater than 2000 were replaced by lowercase n's. Because Mfold is not able to pair n's, all of those nucleotides are unpaired.

This secondary structure is likely a good model, since few of the less reactive nucleotides are unpaired, and only one structure was predicted by Mfold.

Further Research

Various experiments are needed to investigate whether the secondary structure constructed by the Mfold program is accurate. Such experiments could switch two paired nucleotides (base covariation) or remove an unnecessary section of the ribozyme (truncation).

If the predicted structure is correct, these mutations will not affect the function of the ribozyme. If the predicted structure is incorrect, the mutations will prevent triphosphorylation.

1. Moretti, J.E. & Muller, U.F. A ribozyme that triphosphorylates RNA 5'-hydroxyl groups. *Nucleic Acids Res* **42**, 4767-78 (2014).
2. Mortimer, S.A. & Weeks, K.M. A fast-acting reagent for accurate analysis of RNA secondary and tertiary structure by SHAPE chemistry. *J Am Chem Soc* **129**, 4144-5 (2007).
3. Zuker, M. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res* **31**, 3406-15 (2003).
4. Pasek, M.A., et al. Production of potentially prebiotic condensed phosphates by phosphorus redox chemistry. *Angew Chem Int Ed Engl* **47**, 7918-20 (2008).
5. Pasek, M.A., Herschy, B. & Kee, T.P. Phosphorus: a case for minerals-organic reactions in prebiotic biochemistry. *Orig Life Evol Biosph* **45**, 207-18 (2015).