OBSERVING PUTATIVE RNA STRUCTURES THAT RESIST XRN1 DEGRADATION

Abstract

A critical aspect of RNA biology is observing and experimenting with secondary and tertiary RNA structures that provide unique functions within the cell. One of these RNA structures is the pseudoknot. Specifically, this research will be aimed to test in-vitro transcribed putative XRN1-resistant xrRNA structures known as pseudoknots within mRNA transcripts. This study will utilize the enzyme XRN-1 and the complementary DNA to produce the mRNA transcript. The pseudoknot's ability to stop degradation of the mRNA transcript will be assessed through denaturing polyacrylamide gel electrophoresis (dPAGE) analysis. Even though, the results of this experiment have not yet manifested. There are a few possibilities of what the results of the experiment might be: 1) Definitive proof of the RNA transcripts resisting XRN1 degradation 2) XRN1 completely degrades the RNA transcripts and there is no sign of 5' truncated products. Understanding and researching such structures could reveal an unexplored facet of gene expression/regulation.

Significance

Ribonucleic acids (RNA) are polymeric molecules that are essential for life. While best known for its role in protein synthesis, RNA also plays an important role in cellular regulation. Generally, RNA achieves this regulation through the threedimensional structure it folds into. One class of these structures are exoribonuclease resistant RNA structures (xrRNAs), which are resistant to exoribonucleolytic decay. xrRNAs came to prominence due to their discovery and critical role in Flaviviruses, where an xrRNA structure plays an essential role in viral infection. XRN1, an endogenous exoribonuclease found in humans, normally plays an important role in RNA degradation, but in the case of Flaviviruses, only partial degradation occurs due to the xrRNA structure (Geisler, 2012). This leads to the production of biologically active sub-genomic RNA (sqRNA) sequences that are necessary for transmission between mosquitoes and humans (Steckelberg, 2018). Due to the observed importance of xrRNAs in flaviviruses, it is of interest to determine if they are present in other classes of organisms as well. An essential component of the flaviral xrRNA is the presence of a pseudoknot, which, as seen in Figure 1, is a type of RNA structure that involves the base pairing of a single stranded RNA to a complementary sequence located on the initial mRNA structure (Brierley et al., 2007). Consequently, this research will be aimed at identifying a novel pseudoknot in Arabidopsis, which we will confirm as an xrRNA by treatment with XRN1.

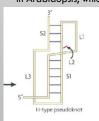


Figure 1. The figure shown above shows a roadkill diagram of a xrRNA structure known as a H-type pseudoknot. As seen in the figure above, a single stranded RNA strand loops back to form a bond with the already base paired RNA secondary structure. Thus, resulting in the formation of a pseudoknot (Brierley et al., 2007).

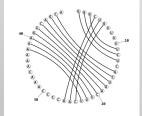


Figure 2. The figure above shows an example RNA sequence, which contains a pseudoknot. A pseudoknot structure can be predicted through the program, ProbKnot, which can show the base pairing interactions between nucleotides.

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Objective

To confirm and observe the pseudoknots capability to resist XRN1 degradation.

<u>Methodology</u>

The start of the research consisted of a hybrid bioinformatic/literature research, which led to the identification of several potential XRN1 resistant pseudoknot structures. The sequences proposed to be tested in this work was derived from a research paper regarding the presence of 5'-truncated, or partially degraded RNAs present in the cell of Arabidopsis thaliana, a species of plant. In the 2012 paper by Nagarajan and co-workers, selectively sequenced free 5' monophosphorylated RNA transcripts were identified. This finding suggests these RNA transcripts were partially degraded by XRN4, which is a plant homolog exoribonuclease of XRN1. Their resistance to XRN4 degradation could represent their resistance to XRN1 decay, though these have not been individually confirmed or investigated.

Based on the RNA sequencing protocols, several XRN4 resistant RNA structures can be inferred. By reading and analyzing Nagarajan's research paper, three potential XRN1 resistant mRNA sequences were extrapolated from the paper: 1) AT4G36920.1 2) AY1G12520.1 3) AT1G30210.1. The paper stated that the mRNA sequences resisted XRN4 decay. Specifically, the wet lab portion will consist of an in-vitro experiment involving purified RNA molecules and XRN1. In a typical RNA experiment, substrate RNAs are prepared from double-stranded DNA templates, which are incubated in a test tube with XRN1. These subsequent RNAs are analyzed by denaturing polyacrylamide gel electrophoresis (dPAGE). Putative XRN1 resistant RNA structures will be identified by the presence of a 5' truncated product apparent in the gel. The XRN1 5' truncated RNA product can be seen in figure 3.

Expected Results

The expected results of this experiment is that the mRNA transcripts will resist XRN1 degradation, which will reveal 5' truncated products in the dPAGE gel.

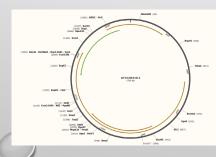


Figure 3. Snapgene of AT1G30210.1



Figure 4. ProbKnot Structure of AT1G30210.1

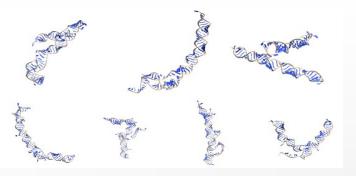


Figure 5. The figures shown above shows the seven 3D models of AT1G30210 using RNAComposer. As seen in the figures above, there are seven separate 3D models, which represent different partitions of AT1G30210.1. The following order is as follows: top left to top right, bottom left to bottom right.

Acknowledgements

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