Structural Characterization of Lupus Antigen-Related Proteins with Solution-State NMR & Electron Microscopy

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Abstract

Type I collagen is the most abundant protein in vertebrates, but it's rate of synthesis is slow, due to the protein's long half-life (50-70 days). However, this rate is increased by several hundred-fold during reparative or reactive fibrosis. The mechanisms responsible for this regulation are complex, but posttranscriptional regulation plays a major role. Post transcriptional regulation is comprised of a series of steps: mRNA transport, stabilization, and translation, which are executed by mRNA binding proteins. While there are over 800 RNA binding proteins only one, La ribonucleoprotein number 6 (LARP6) is involved in type I collaged regulation. It is known that LARP6 binds to the 5' stem loop in sequence manner, recruiting other proteins, including: non-muscle myosin, vimentin, RNA helicase A, serine threonine kinase receptor associated protein, and 25 kD FK506 binding protein, which increase the stability and translatability of type I collagen mRNAs. However, little is known about its actual 3-demensional structure and domain-orientation due to its size, which makes it too large for nuclear magnetic resonance spectroscopy (NMR), yet too small for electron microscopy (EM). Furthermore, the unbonded protein's instability at high concentrations further exacts collecting NMR data. Here, we will utilize a tRNA scaffold to enable to structural characterization of LARP6 via Cryo-EM.

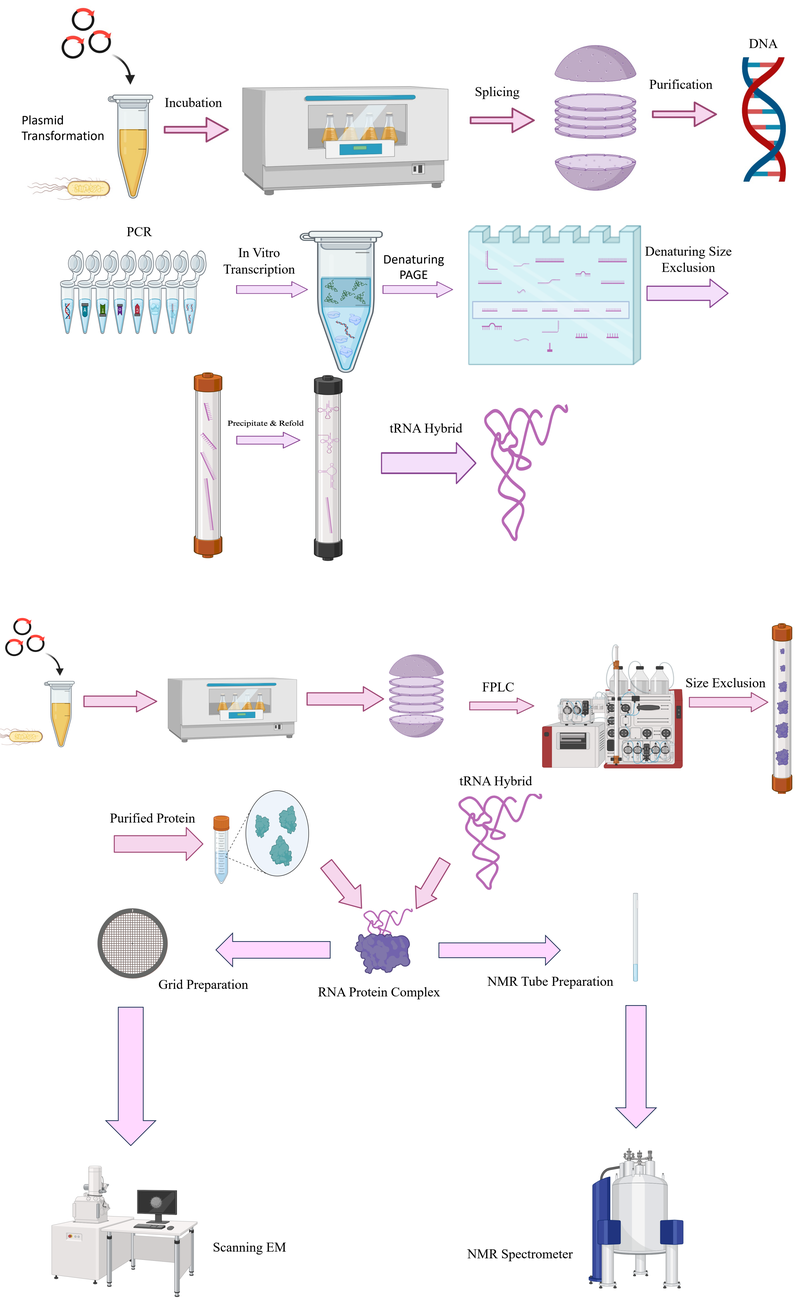
Background

LARP-6 is a human protein responsible for the regulation of collage I (a structural protein found in the skin and fibers of most mammals). It is known that LARP-6 binds to m-RNA, creating an attachment point for other proteins responsible for unfolding the RNA, making its translation more probable. It is also known that high levels of LARP-6 increase the probability of fibrosis. However, little is known about its structure due to its size, which is too large for NMR, yet too small for electron microscopy. In addition, the protein is highly unstable and while unbonded to m-RNA, making it likely that it will precipitate in solution, further slowing its research. This research aims to examine LARP-6 using NRM and electron microscopy, while acquiring further information on the effects it has on the human body.

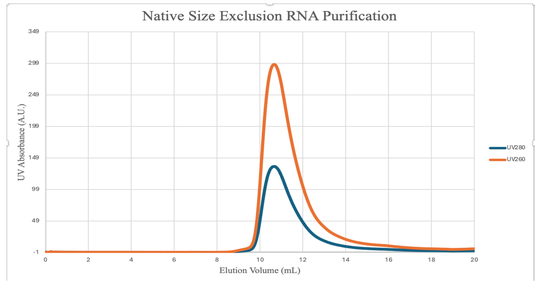
Future Direction/implications

After the promising results from negative staining, the next step is to analyze the sample under Cryo-EM. The presence of the protein demonstrates that the extraction methods are yielding the desired complex. More testing will be required to fully understand the structure of LARP6.

Methods

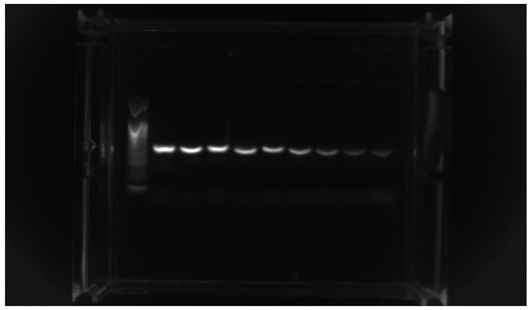


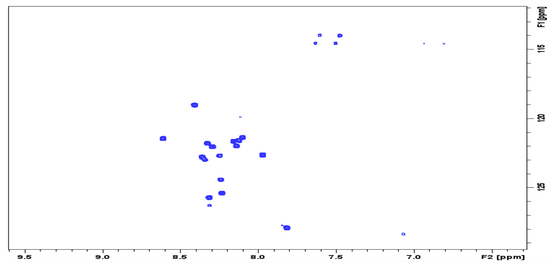
Preliminary Results



The denaturing acrylamide gel allows us to examine the RNA's size. Since the tRNA is unfolded, only the size and charge of the molecule will play a role on how fast it moves. This gel shows RNA from samples of various concentrations, demonstrating that our RNA is the correct length.

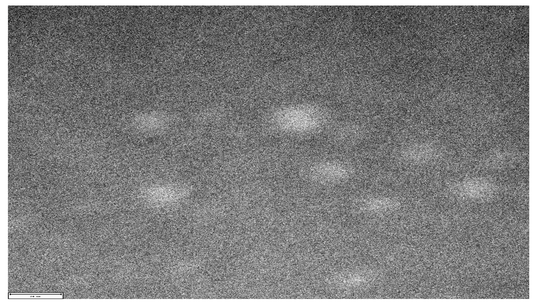
The increased UV absorbance at a wavelength of 260 demonstrates ow that this sample is high in nucleic acids, in this case, RNA.





NMR testing gave inconclusive results, since most of the peaks were missing likely due to molecular weight and aggregation. The peaks shown are likely in the N and C termini, where the protein is flexible.

Negative stain electron microscopy yielded promising results, since a molecules around 10nM in length, the estimated size of our protein RNA complex, are noticeable.



References

•Ricard-Blum S., Ruggiero F. The collagen superfamily: From the extracellular matrix to the cell membrane. *Pathol. Biol. (Paris). 2005;53:430–442. doi: 10.1016/j.patbio.2004.12.024Zhang, Yujie, and Stefanovic Branko. 2016. "LARP 6 Meets Collagen mRNA: Spacific Regulation of Type I Collagen Expression." International Journal Of Molecular Sciences 419.*

•*Kozak M. An analysis of 5′-noncoding sequences from 699 vertebrate messenger RNAs. Nucleic Acids Res. 1987;15:8125–8148. doi: 10.1093/nar/15.20.8125*

•*Zhang, Yujie, and Stefanovic Branko. 2016. "LARP 6 Meets Collagen mRNA: Spacific Regulation of Type I Collagen Expression." International Journal Of Molecular Sciences 419.*

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