Using electron microscopy to visualize the Ornate Large Extremophilic RNA fused to a Group II Intron Ribonucleoprotein

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Abstract

Ornate Large Extremophilic (OLE) RNAs are non-coding transcripts that are mainly found in Gram-positive, extremophilic bacteria. In our experiment, the OLE RNA is fused to the Tel 4h RNA. The Tel 4h protein binds to the Tel 4h RNA and forms the ribonucleoprotein, which can retrotranspose into a target DNA. To isolate the TelHydraOLE ribonucleoprotein, we used desthiobiotin-labeled target DNA and avidin resin. Previous research has suggested that the OLE RNA is recruited with an OLE associated protein to the membrane when there is near toxic levels of alcohol present in the cellular environment. To learn more about what role OLE RNA has in mediating alcohol toxicity, we are using electron microscopy to determine the structure of OLE RNA in order to infer its function. We will utilize electron microscopy to obtain high resolution images, and eventually create a 3D structure of the OLE RNA.

We then did an ribonucleoprotein (RNP) Assembly using a desthiobiotin labeled DNA primer which binds the TelhydraOLE RNP. We bound the RNA, protein, and primer together in a retrotransposition reaction at 50°C. Once the RNP was assembled, we added avidin resin to the sample and let them bind for about an hour. After washing the RNP several times, we eluted the RNP from the resin with a buffer saturated with biotin. We concentrated the RNP by centrifuging it in a 100 kilodalton filter and measured the concentration once the volume was about 25 uL. We then diluted it down to three different concentrations to prepare grids for electron microscopy. Then we prepared four copper grids with the diluted samples of TelhydraOLE RNP and negatively stained them. We went to UC Irvine to use their electron microscope to obtain images of the OLE RNA in the grids.

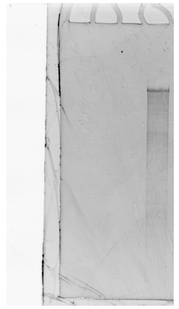
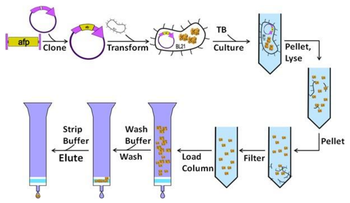


Image of Gel with 1 ug of TelHydraOLE RNA on it

Methods



Protein Purification Process

We prepared two 2 L and one 100 mL Luria-Bertani (L.B.) solutions and then we autoclaved these solutions to make sure the solutions are sterile. Next, we added carbenicillin and Tel 4h protein cells from a glycerol stock to a 100 mL L.B. starter culture and let it grow overnight at 37 °C. We added carbenicillin to the two flasks of 2 liters of L.B. and also added 20 mL of the starter culture (which was made the previous day) to both these flasks. We looked at 1 mL samples from each of the flasks and measured their optical densities, using a blank of 1 mL of L.B. with no cells. Once we reached an optical density of about 0.7, we added 2 mL of 1 M IPTG in order to induce protein production in the cells. Then, both the flasks were incubated again at 22°C.

We then spun down the cells formed in the flasks in centrifugal bottles to pellet the cells and then we dumped out the supernatant. We resuspended the cell pellet in lysis buffer in order to keep the proteins within the cells well folded. We then transferred the resuspended cell pellets to a 60 mL beaker and kept it on ice. The beaker with the cells was then brought to a sonicator in order to lyse the cells. The cell suspension was then transferred to two 50 mL conical tubes and then centrifuged. While that was spinning, we prepared 4.3 mL of nickel resin resuspension by washing the resin with lysis buffer three times. We separated the supernatant from the pelleted cell debris by pipetting off the supernatant and adding it to the resin. We put the tube with resin and the soluble fraction of cells in a cold room at 4°C. The nickel resin was expected to bind to the protein's polyhistidine-tag. After an hour in the cold room, the excess supernatant was tossed. The resin was transferred to a column and was washed with seven different buffers. Then, we added the elution buffer, which disassociates the protein from the resin due to a high concentration of imidazole.

We saved 25 mL of the eluted protein and split the sample between two 50 kilodalton centrifugal tubes. Finally, we washed and centrifuged the protein multiple times to get rid of the imidazole. We then prepared 2 SDS-PAGE gels to estimate the concentration of the Tel 4h protein and evaluate the purity of protein purification samples.

In order to prepare the TelhydraOLE RNA, we made a 2 mL transcription mixture that included digested DNA. After the transcription reaction was complete, we added DNase and proteinase K to degrade the protein and DNA. The RNA was then washed multiple times in a 100 kilodalton centrifugal filter to put the RNA in a new buffer. We then made a 4% PAGE gel and prepared RNA samples in 40% formamide. We put the samples on the heating block for 2 minutes and then snap cooled them on ice. The samples ran on the gels for 2 hours at 25 Watts and then the gel was taken down and stained with ethidium bromide for 10 minutes. The gel was then wrapped in plastic and shot with UV light to take a picture of the TelhydraOLE RNA.

Relevant Images

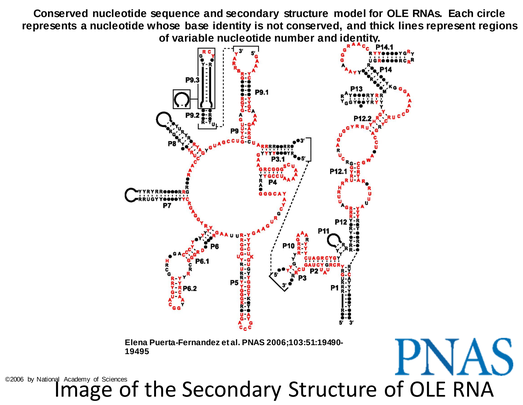
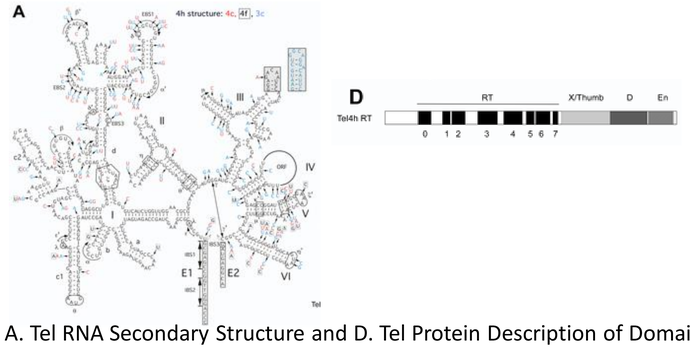
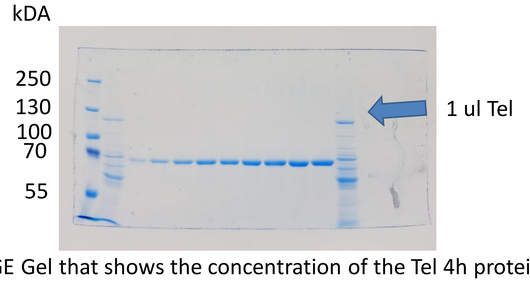


Image of the Secondary Structure of OLE RNA



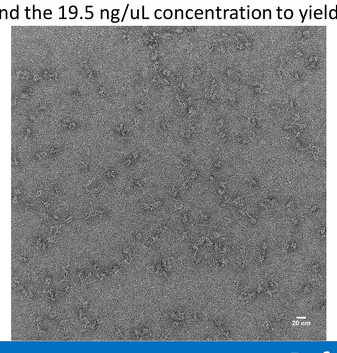
A. Tel RNA Secondary Structure and D. Tel Protein Description of Domains



SDS-PAGE Gel that shows the concentration of the Tel 4h protein

Analysis and Conclusion

We found the protein yield of the Tel 4h protein, after isolating it in the protein purification process, to be 648 ug protein. The RNA yield, after purifying for the RNA, came out to be 1432 ug. The Ribonucleoprotein complex was concentrated down to 39 ng/uL and then diluted down to these three different concentration to be applied to copper grids; one being 15.6 ng/uL, one being 25 ng/uL, and two being 19.5 ng/uL. We found the 19.5 ng/uL concentration to yield images of homogenous particles.



Electron Microscope Image of the TelHydraOLE RNP

References

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