Role of Dimerization in Retrotransposition of Group II Introns

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Abstract

Mobile genetic elements are able to insert themselves into the genome of their host organisms. There are two main classes of mobile genetic elements: transposons and retrotransposons, also known as retroelements. The main difference between transposons and retrotransposons is that retrotransposons have an RNA intermediate before insertion into the host cell's DNA. Retroelements are able to insert themselves into DNA through a copy and paste mechanism known as retrotransposition. To study the structural basis of retrotransposition, I am working with a group II intron from a thermophilic cyanobacterium. It has been proposed that an important feature of the mechanism of retrotransposition is the dimer. However, the identity of the dimerization has remained elusive in both biochemical and structural studies. From previous cryogenic Electron Microscopy experiments, a dimer was observed and initially characterized, however, there were too few particles to obtain a 3D reconstruction of high enough quality to correctly observe the function. Specifically, I am performing crosslinking experiments to obtain a higher concentration of dimers in solution. This will hopefully lead to a higher particle number in cryo-EM experiments. With this characterization, I hope to obtain a better understanding of the structure of the dimer and its and function in the process of retrotransposition.

Mechanism of Retrotransposition

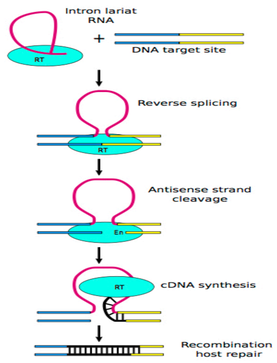
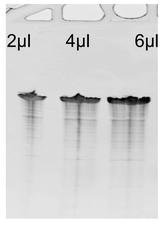


Fig 1. Mechanism of retrotransposition

Retrotransposition has 4 main stages: the first is reverse splicing. Reverse splicing is performed by the RNA and consists of the ribozyme inserting itself into the host cell's DNA. Secondly, the endonuclease domain cleaves the bottom strand creating the free 3'-OH needed for cDNA synthesis by the reverse transcriptase(RT). Lastly, the RNA gets replaced by host cell repair mechanisms

Methods

For the purpose of producing RNA, the *In Vitro* transcription method was used. In this process, the pUC57 plasmid was transformed into E. coli cells, which were then plated on agar with LB broth and carbenicillin, and allowed to grow. The pUC57 plasmid has a sequence which encodes antibiotic resistance, this was to ensure that only the cells that had transformed the plasmid would survive and the others would be killed by the antibiotic carbenicillin. Once a specific colony was chosen, the cells were then grown in LB broth with carbenicillin. To purify the plasmid from the cells, a Maxi-prep was performed to lyse the cells and separate the DNA. Next, the plasmid was linearized to obtain the specific sequence needed for the RNA. Lastly, the RNA was synthesized from the DNA template using *in vitro* transcription. A polyacrylamide gel was also run of the RNA to observe purity.



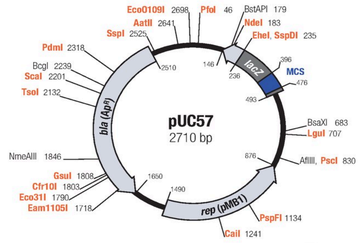


Fig 2a. Denaturing page gel of *in vitro* transcribed RNA **Fig 2b. pUC57 plasmid**

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Recombinant protein purification was the method used to obtain the intron encoded protein (IEP). LB broth was inoculated with E. coli cells from a glycerol stock containing the necessary protein. A lactose imitator known as Isopropol β-D-1-thiogalactopyranoside(Iptg) was then added to allow the lac operon of the cells' genomes to activate and transcribe the RNA needed for the protein sequence. The cells were then left to grow for 48 hours to translate and synthesize the protein. Following, the cells were harvested using centrifugation and the cell pellets were resuspended in lysis buffer. The cells were lysed using a probe sonicator. The lysate was then allowed to mix with Ni-NTA resin to allow the N-terminal 6x-His tag of the protein to bind the Ni^{2+}. The resin was then loaded onto a column and the resin was washed with several column volumes of wash buffer to remove any contaminating proteins. A solution of 250mM imidazole was used to elute the protein from the nickel resin once the lysate had been purified. The protein was then filtered alone to attain the most pure sample of the protein and to buffer exchange the imidazole away. To check the purity of the protein, an SDS page gel was run. As an activity check, a splicing gel was run.

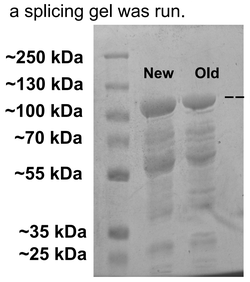

Fig 2c. SDS Page Gel

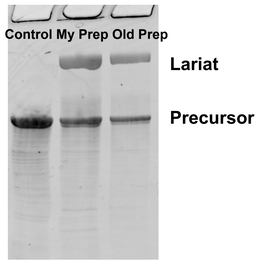

Fig 2d. Splicing Gel

The ribonucleoprotein(RNP) bound to a DNA complex was purified using a biotinalated oligo using a batch purification method. First the RNA protein complex was assembled in the same conditions used for splicing. The biotinalated DNA oligo was then added and left to react for 6 minutes at 50 ºC. The RNP-DNA complex was bound to avidin resin . The resin was washed 5 times and was eluted with a solution containing saturated biotin. The eluent was then mixed with various concentrations of formaldehyde or glutaraldehyde as cross-linking agents, and left to sit for 1 hour. Negative stain grids were then prepared using a 2% uranyl acetate.

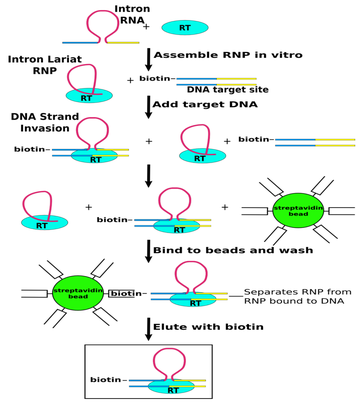

Fig 2e. Biotin RNP purification

Results

During the RNP-DNA protein purification, a concentration too low to work with was first observed. Subsequently, a concentration of the sample was performed using a 30 kDa cut off filter. The concentration increased initially, but during the final filtration returned to the pre-filtered concentration. The Negative Stain grids were still prepared and observed using an Electron Microscope. No particles were observed on the grids.

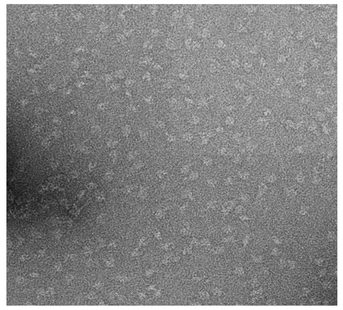

Fig 3a. Negative Stain Grid of RNP-DNA

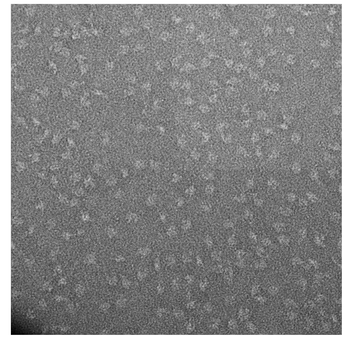

Fig 3b. Negative Stain Grid of RNP-DNA

Analysis and Conclusions

In future experiments, a higher concentration of the particles will attempted to be attained. Furthermore, more cross-linking experiments will be performed with varying concentrations and methods of cross-linking. Ultimately, once the proper cross-linking conditions have been determined between the retroelements, higher resolution images will hopefully be obtained. With these images, a 3D structure of the dimer will be constructed for the eventual purpose of determining the role of the dimer configuration. This information can then be used to further study group II introns and other retrotransposons.

References

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