Metazoan mitochondrial alanyl-tRNA synthetases

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Introduction

Animal mitochondrial tRNAs are not well-defined. Their tRNAs are highly diverged in both sequence and structure from those found elsewhere in evolution. The loops and stem size in their predicted secondary structures are variable and the conserved nucleotides are often not present. The tRNAs are recognized and charged by nuclearly-encoded mitochondrial aminoalanyl-tRNA synthetase.

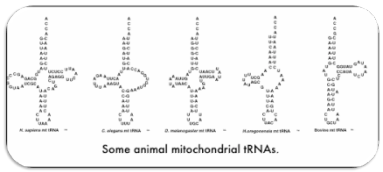


Fig. 1 Some animal mitochondrial tRNAs. Animal mitochondrial tRNAs are diverged in sequence and structure. *Taken from Jolene and Nakati's PowerPoint presentation.

Our work focused on the interaction between different animal mitochondrial alanyl-tRNA synthetases and their tRNA substrates. We focused mainly on C.elegans tRNA and human mitochondrial tRNA, although our initial experiment was on E.coli. We tested E.coli to see if our method worked. We hypothesized that the differences in tRNA sequence and structure of animal mitochondrial tRNAs require a different mode of binding and recognition than that used by typical alanyl-tRNA synthetases.

Assay

To locate the recognition site of the tRNA with its alanyl tRNA synthetase, we used the footprinting assay. The footprinting assay shows where the tRNA is protected form RNase cleavage.

RNase is an enzyme that cleaves (hydrolyzes) tRNA, often with affinity for specific bases. The RNase that we used included RNase T1 (cleaves at single stranded Gs), RNase V_1 (cleaves double stranded RNA), and RNase A (cleaves at single Cs and Us).

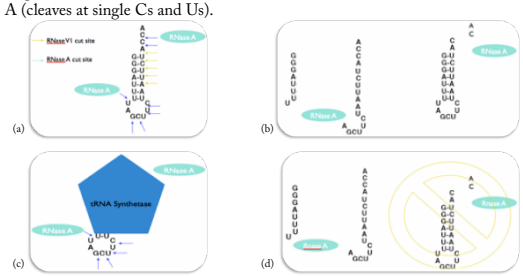


Fig. 2 The process of the footprinting assay. (a) The RNase cleaves the tRNA at the predictable places. (b) These are the possible cleavage sites in the tRNA. (c) When the synthetase bounds to the tRNA, the RNase can no longer cleave the tRNA at that particular site, and only one cleavage site is possible (4). *Taken from Jolene and Nakati's PowerPoint presentation.

Methods

The plasmids that we used were created by Joe Chihade. We used enzyme BSTN1 to digest the plasmids . We proceeded with transcription and inserted the hammerhead ribozyme sequence, which consisted of many single Gs, in front of the wanted gene.

To locate the RNA on the gels, we used a technique called UV shadowing. We placed the gel on a phosphorus florescent TLC plate. When we shine the UV light on the plate, it excites the plate. This caused the RNA to absorb the light and cast a shadow on the plate.

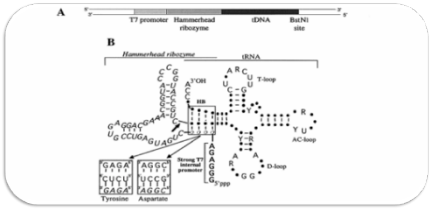


Fig. 3 Hammerhead ribozyme. Fechter, Pierre. 1998. The hammerhead ribozyme is an RNA enzyme that catalyzes reaction with the ability to cut itself from the tRNA. It provides animal mitochondrial tRNAs with the necessary sequence of Gs to start the transcription process.

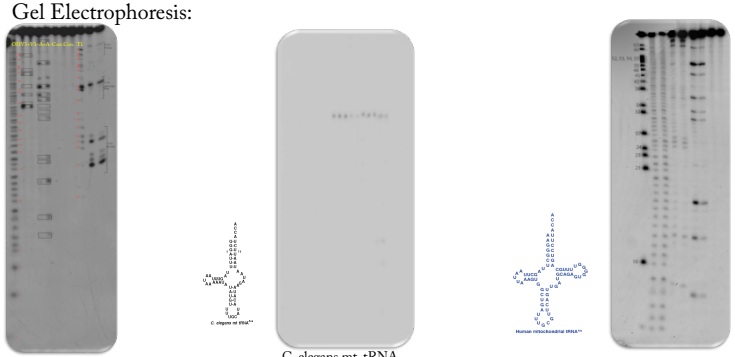
After the transcription process, we ran a purification gel and extracted the RNA from the gel using electrophoresis. We denatured the tRNA and labeled the 5' end with radioactive ^{32}P. We cleaned and concentrated the radioactive tRNA and ran it on a small analytical gel. We soaked the radioactive tRNA gel pieces in the Crush and Soak solution and let it rotate overnight. We used a spin column to extract the radioactive tRNA solution.

We proceeded with the footprinting assay. We performed the ASSAY program on the PCR machine to renature the radioactive tRNA. We split the renatured tRNA into five tubes: RNase A+, A-, V_1+ , V_1- , and a control (+ or – indicates the presence or absence of synthethase in the final reaction). We used the 5' end labeled tRNA to run a T1 digest to serve as a ladder and OH ladders (incubated at two different time intervals) treated with base hydrolysis, so we could see some pieces of every length. We precipitated the digested tRNA except for the T1 digest and the OH ladders.

We loaded all the samples onto a sequencing gel. We ran the gel and exposed it onto a phosphorus screen. We used the Storm scanner to scan the phosphorus screen.

Results

Gel Electrophoresis:



E.coli C. elegans mt. tRNA Human mt. tRNA

Fig. 4 Sequencing gels of the E.coli, C.elegans mt. tRNA, and Human mt. tRNA. The box indicates that there is a difference between the two bands at the particular nucleotide.

VMD Images:

After we identified where on the tRNA the nucleotides were protected from the RNase cleavage, we plotted those protected sites using a software called Visual Molecular Dynamics (VMD). VMD generated a three-dimensional image of the tRNA. This allows us to see if the protected sites are located in a particular location on the tRNA.

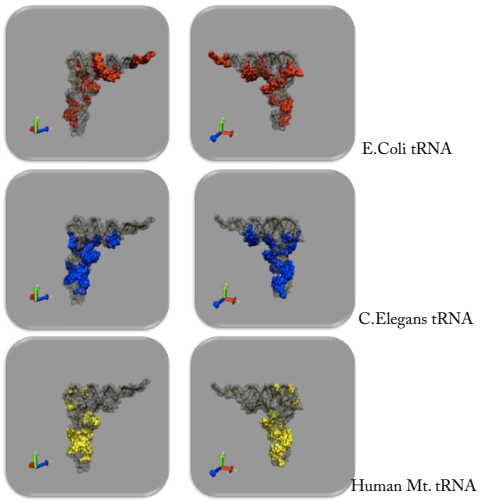


Fig. 5 3-D images of the tRNAs. The colored areas on the tRNA correspond to the sites where the synthethase interacts with the tRNA. These are the protected sites of the tRNA from RNase cleavage.

Summary

Animals mitochondrial tRNAs are different in their structure and sequence. To better understand the basis of specific recognition of mt. $tRNA^{Ala}$, we used the footprinting assay. This allows us to identify the site where the enzyme interacts with the tRNA.

Future Endeavors

We hope to analyze the data that we have collected and conduct more experiments on other animal mitochondrial tRNA and their alanyl-tRNA synthetase. Through this we can better understand the basis of specific recognition of mt $tRNA^{Ala}$.

Acknowledgements

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