Retinoid x Receptor Alpha DNA Binding for Mitochondrial Transcription

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Introduction

Mitochondria are important organelles in eukaryotic cells due to their role in metabolism. They also have their own genome (mtDNA) which encodes many of the protein subunits within the oxidative phosphorylation (OXPHOS) pathway. The remaining protein subunits are encoded in the nuclear genome, translated in the cytosol and imported into mitochondria. Transcriptional coordination between these two genomes to produce functional OXPHOS complexes is critical to maintaining cellular homeostasis.[1] A major control point is transcription, and this process requires regulatory proteins that determine the expression of these genes.[1] Retinoid X receptor alpha (RXRα) is a protein that has many functions in human cells and is a ligand activated nuclear receptor.[2] It can also translocate to the mitochondria where its purpose is unknown.[3] **The goal of this project is to determine the role of RxRα as a mitochondrial transcription factor.** We have expressed and purified the RXRα protein, and then also assessed its DNA binding affinity with various mtDNA sequences. We concluded RXRα binds as a dimer with space between the binding sites. Our aim is to investigate RxRa binding the mtDNA using atomic force microscopy (AFM). In doing so we will better understand the mechanisms that are involved in mtDNA transcription. If RxRα is a transcription factor that is another protein that we can regulate to increase or decrease transcription. Being able to increase or decrease transcription in mitochondria is important as mitochondrial dysfunction causes a wide array of diseases and plays a part in aging.

Methods

Bacterial Transformation & Protein Expression

Plasmid DNA[4] (Figure 2) were extracted via a Zymo Research mini-prep kit then transformed into to competent *E. coli* cells via heat shock. Cells were cultured in LB media for 2 days at 37°C with IPTG (0.1 mM) used to express the RxRα protein.

Bacterial Lysis

Cells were pelleted by centrifugation. Buffer with Triton X-100 was added to the cells, vortexed and sonicated, then centrifuged again to separate out protein from other cell debris.

RxRa Protein Purification

Protein was purified using GST affinity column chromatography (Cytiva) with PBS and then 10 mM glutathione to elute the protein. The protein was concentrated by filter centrifuging (Amicon).

dsDNA Oligo Probe Formation

DNA oligo probes were created by combining the forward and reverse oligos, boiling, then letting it cool for 30 minutes to allow the dsDNA to anneal. The DR1 repeats were separated by 2 bases, and 5 bases (Figure 3), and third probe was tested with a single DR1 sequence (TGGTCA).[5]

DNA/Protein Complex

Electrophoretic mobility shift assay (EMSA) was used to assess the protein/DNA complex. Reactions included purified protein, BSA, 2x reaction buffer, DNA. Reactions were visualized on native gels.

Results

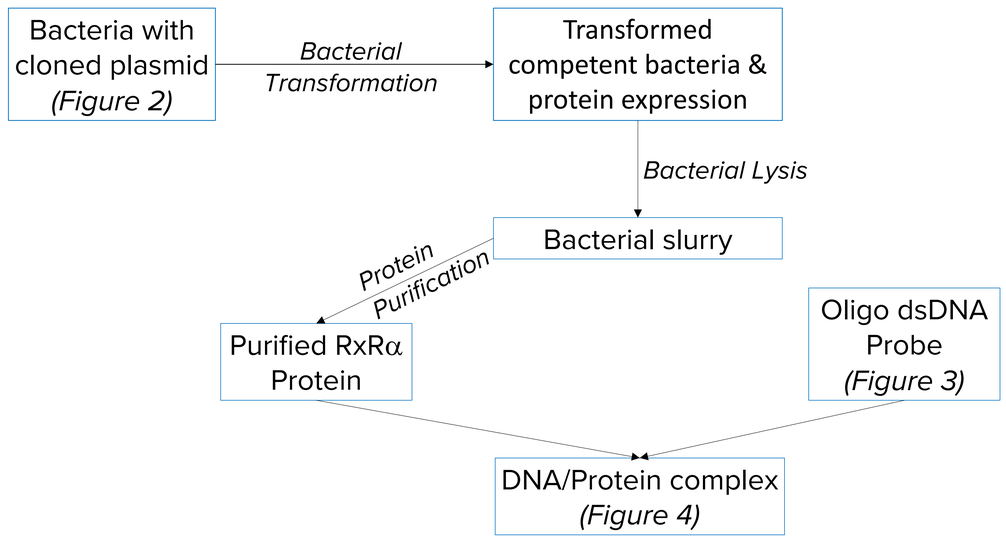


Figure 1. Procedural Flow Chart.

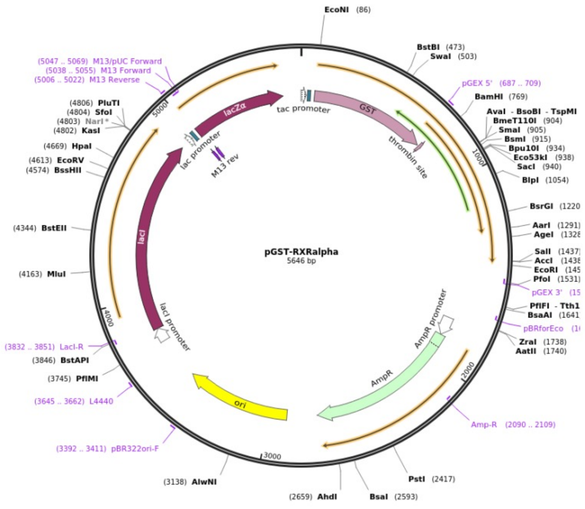


Figure 2. RxRα Expression Plasmid. pGST-RXRalpha was a gift from Bert Vogelstein (Addgene plasmid # 16547).

Figure 4. DNA/Protein Complex. EMSA was used to assess RxRα binding to the DR1 repeats separated by 2 and 5 nucleotides. This image shows RxRα binding to DR1 repeats separated by 5 nucleotides (2 ug probe). The RxRα concentration represents starting with 10 ug protein followed by a 1:5 serial dilution. Samples were run on a native acrylamide gel and visualize with GelRed.

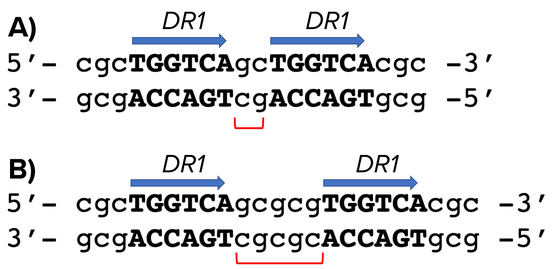
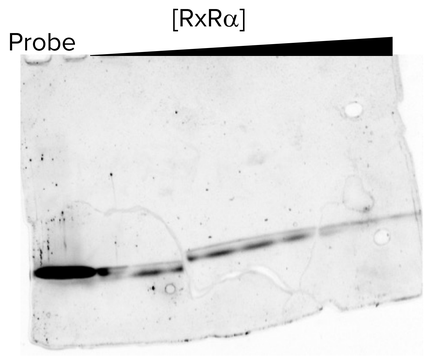


Figure 3. dsDNA Oligo Probe. Two double stranded DNA probes were formed to test the DNA/Protein complex. Both contain the DR1 (direct repeat 1) sequcen in parallel. A) DR1 repeats separated by 2 nucleotides, B) DR1 repeats separated by 5 nucleotides.



Conclusions & Future Directions

- **RxRα was expressed and purified**
- **RxRα binds to DNA with palindromic sequences separated by 5 nucleotides**

Analyze the DNA/Protein complex with an AFM

Use RXRα in the in vitro transcription reaction:

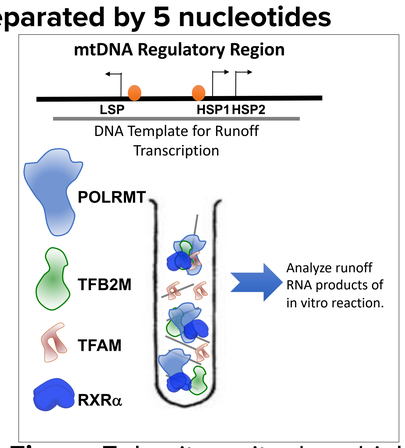


Figure 5. In vitro mitochondrial transcription assay.

References

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