Is RxRα a mtDNA transcription factor?

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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. A major control point is transcription, and this process requires regulatory proteins that determine the expression of these genes. Retinoid X receptor alpha (RXRα) is a protein that has many functions in human cells and is a ligand activated nuclear receptor. It can also translocate to the mitochondria where its purpose is unknown. 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

Plasmids were extracted via a Zymo Research Mini-prep Kit then introduced to the competent cells via heat shock, then incubated for 2 days in IPTG cultures.

Bacterial Lysis

Incubated cells were pelleted by centrifugation, lysed and resuspended in SDS page and centrifuged again. Triton X-100 was added and the cells vortexed the sonicated on ice to separate out protein from other cell debris.

Protein Purification

Protein was purified use a GST affinity column chromatography by cyteva. The protein was then purified by centrifuging the elution samples into one tube.

DNA Oligo formation

Three different palindromic DNA oligos were created by taking the forward and reverse oligos and boiling them over a beaker to synthesize the oligos, then letting it cool for 30 minutes. The palindromic sequences were separated by 0 bases, 2 bases, and 5 bases

DNA/Protein complex

A serial dilution was set up using BSA, 1ug/uL protein, dH_2O, and 2x reaction buffer, DNA was added to each well and then a gel was run with all solutions, the best looking gel is shown in Figure 3.

Results

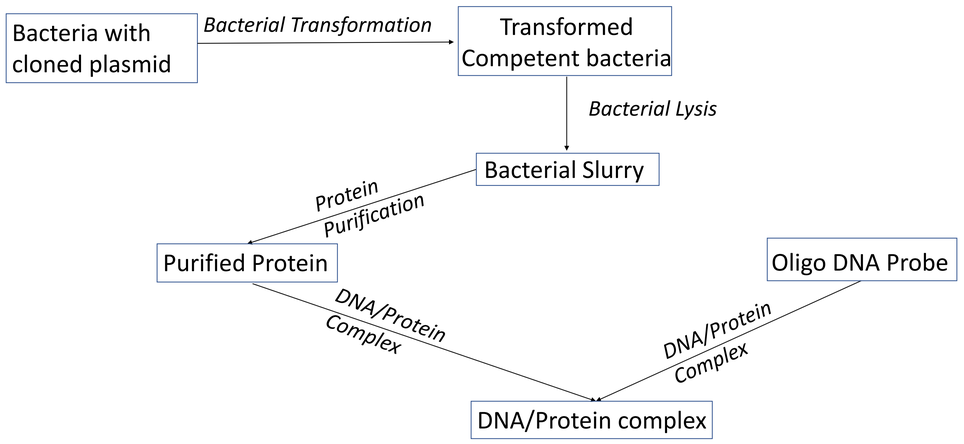

Figure 1. Procedural Flow chart

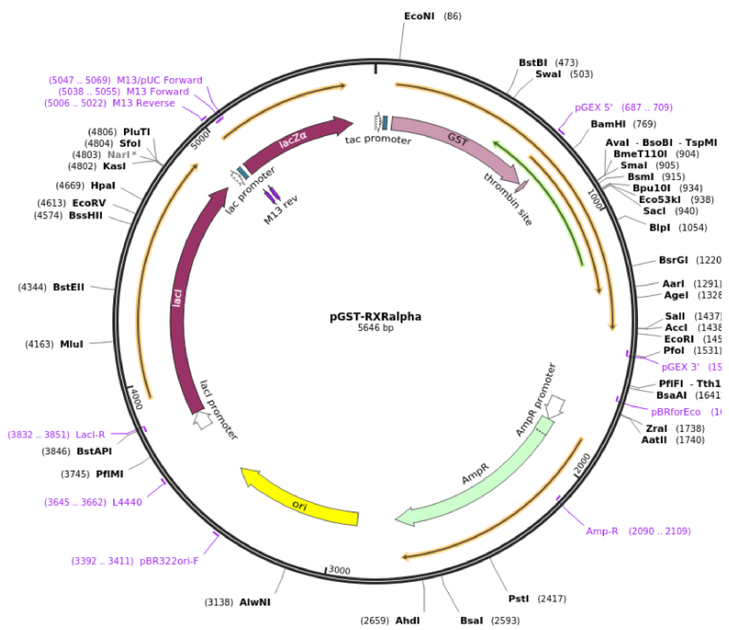

Figure 2. Bacterial Plasmid

Conclusions & Future Directions

- **RxRα binds to DNA with palindromic sequences separated by 5 nucleotides**
- **Want to analyze the DNA/Protein complex with a Atomic Force Microscope**

References

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2. Last name. (Year) Title, *Journal*, Vol, Pg.
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Acknowledgements

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