Identification of Protein Interactions for the Mitochondrial Transcription Factor TFAM and Mutants

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Introduction

Mitochondria are key organelles in eukaryotic cells for their role in metabolism and other biosynthetic pathways. They play a key role in the production of ATP via oxidative phosphorylation (OXPHOS). Over eighty proteins make up the various OXPHOS complexes, (Bestwick, 2013) several of which are encoded by the mitochondrial genome (mtDNA). Mitochondria have double stranded circular DNA molecules that have 37 total genes and no introns encoding OXPHOS complex subunits, tRNAs, and rRNAs (Alvarez, 2008). Within mitochondria the processes of transcription and translation take place to generate these important OXPHOS subunit proteins. During the process of mitochondrial transcription, the transcription factor TFAM (transcription factor A, mitochondria) is important in promoter regulation. TFAM activates transcription at both the light strand promoter, and the heavy strand promoter (Ngo, 2014). TFAM itself is a multifunctional mitochondrial protein in that it binds both specific (promoter) and nonspecific mtDNA sequences. At promoter sequences the protein causes a U-turn in the DNA, while less dramatic bending takes places when bound nonspecifically. TFAM has two high mobility groups that help in the process of making U-turns in the DNA (Ngo, 2014). **Our aim is to identify novel interacting proteins with TFAM using a yeast-two-hybrid model**. Additionally, we are interested in determining if there are changes in the protein interacting partners in mutant forms of TFAM. Specially, two point mutations in the TFAM gene have been linked to late onset Alzheimer's disease, S12T and P178L. The mutation S12T has a nucleotide change of a G to C which causes an amino acid change from a Ser to Thr, and the P178L has a nucleotide changed from a C to a T which causes an amino acid change from a Pro to a Leu (gene cards/ NCBI). *Biochemical and genetic techniques are being used to identify and characterize changes in protein interaction partners as a result of these mutations.*

Methods

TFAM gene cloning:

- PCR was used to amplify the human TFAM gene
- NcoI and SacI restriction enzymes were used to prepare the amplified gene and plasmid (pGEM-T Easy, Promega)
- TFAM gene then ligated with the plasmid
- Restriction digest and sequencing were used to confirm cloning of the TFAM gene in the pGEM-T Easy plasmid

Generation of library DNA:

- RNA was isolated from human liver cells (HepG2 cells)
- cDNA was generated using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems)
- cDNA within the size range of 2500-400 bp was isolated to generate the yeast-2-hybrid library

All DNA products were analyzed using a 1% agarose gel with electrophoresis, and visualized using GelRed (Biotium).

Results

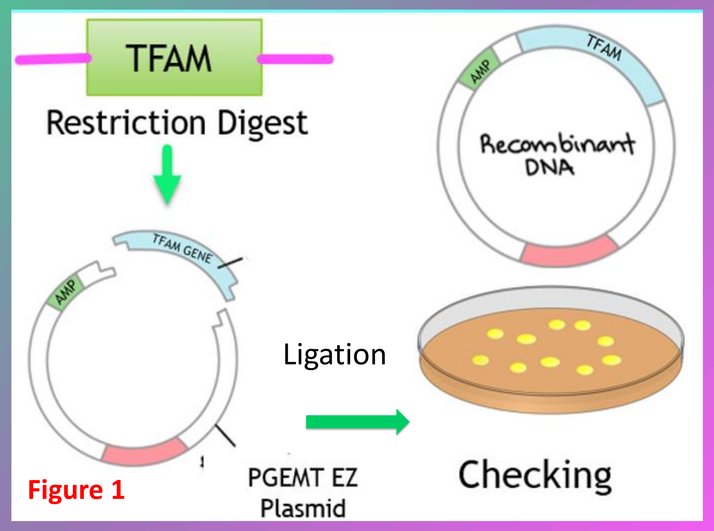

Figure 1

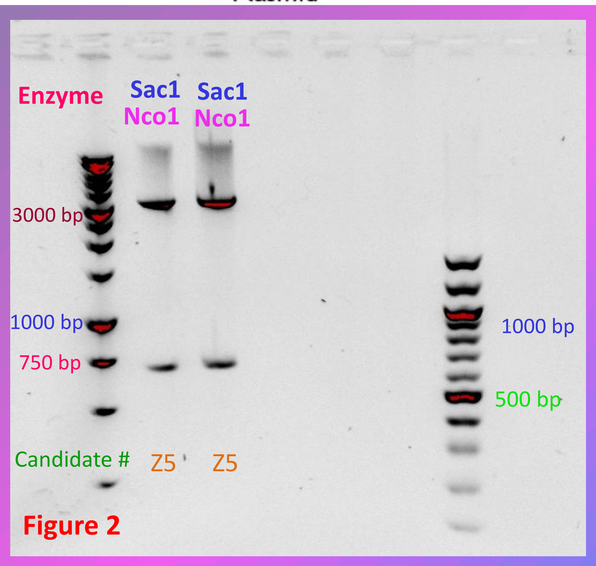

Figure 2

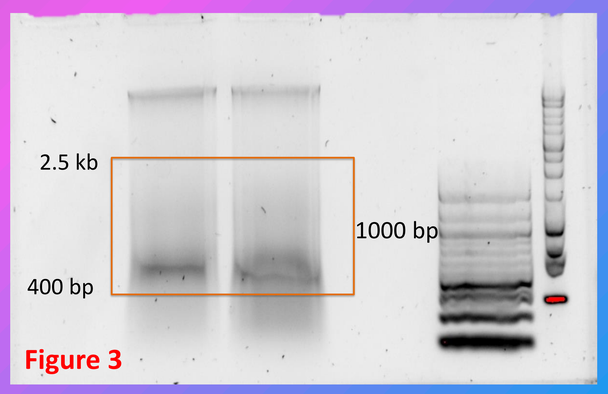

Figure 3

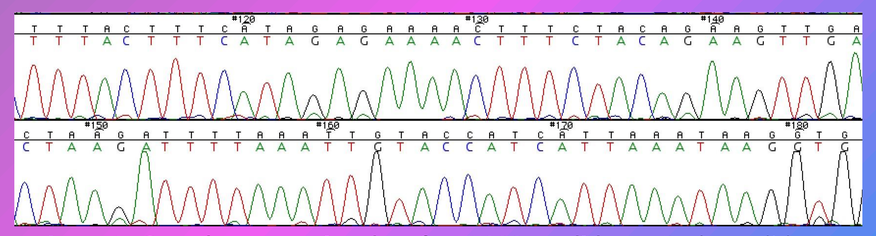

Figure 4. Sample sequencing data for TFAM candidates. Both candidates were sequenced in the forward and reverse directions using M13 primers. Sequencing was performed by Eurofins Genomics (Louisville, KY). Sequencing data as shown was analyzed and compared to the known sequence of TFAM Sequencing data positively confirmed both candidates as containing the human TFAM gene.

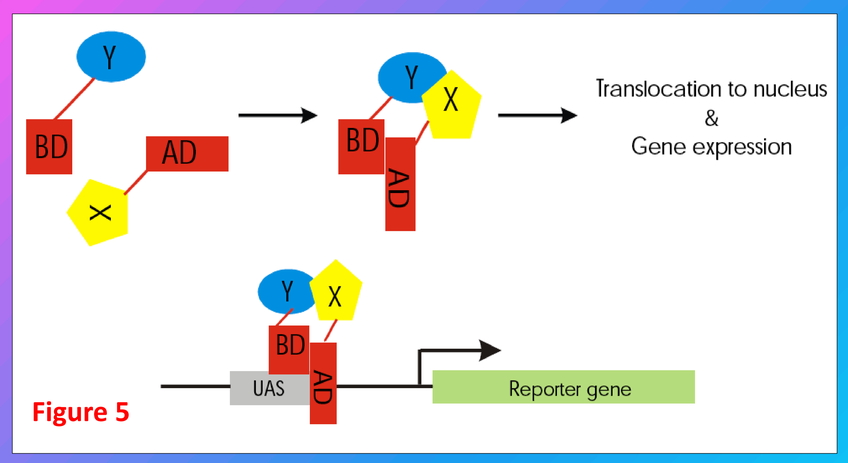

Figure 5

Conclusions & Future Directions

- Site Directed mutagenesis to create the two point mutations that are Linked to Alzheimer's
- Interaction Screen using a yeast 2 hybrid model

References

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Citations for Images

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