Investigating Sequence Variation Effects on Shine Dalgarno in Tau

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Background

Alzheimer's Disease

- Insoluble Fibrous Aggregates
- Lesions on nerve cells composed of Paired Helical Filaments
- Paired Helical Filaments composed of microtubule binding protein Tau

Tau

- What drives aggregation?
- Biological Tau material needed for research
- Recombinant Tau production in E.coli cells

Recombinant Protein

- Production of mass amounts of Tau isoforms in E. coli
- Subject to E. coli cell machinery and regulation
- Suboptimal truncated protein products

Shine Dalgarno

- AGGAGG sequence
- Interacts with 16s rRNA to anchor translation initiation
- Allows start codon to be selected and initiation to begin

Problem Definition

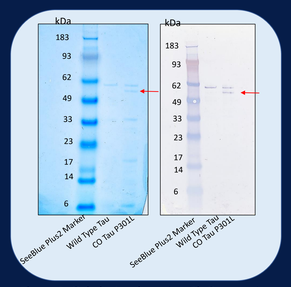

Figure 1. Gel electrophoresis with Coomassie staining and Western Blot analysis of WT Tau and CO Tau P301L

- *Problem*: Protein truncation in CO Tau P301L (red arrows)
- Shine Dalgarno and cryptic Met31 within sequence
- *Purpose*: investigate the sequence components that influence ribosomal binding at SD region

Experimental Strategies

Strategy 1: Mutations in Shine Dalgarno Region
What: Changes within 7 nucleotide region between AGGAGG and cryptic Met 31 in Wild Type Tau sequence.
Goal: To induce truncation in Wild Type Tau

Strategy 2: Site Directed Mutagenesis (SDM)
What: Agilent Quickchange II SDM Kit and 4 designed primers (each 6 codons) identifying from nucleotide 76 to 91 in Wild Type Tau.
Goal: To determine the effects of similarity to 16s rRNA and the identity of the cryptic start site on ribosomal binding

Strategy 3: G Block Insertion
What: Insertion of 150 nucleotide G blocks changing CO Tau P301L upstream, downstream, or within SD region
Goal: To determine if changes in different regions effect ribosomal binding and truncation

Strategy 1

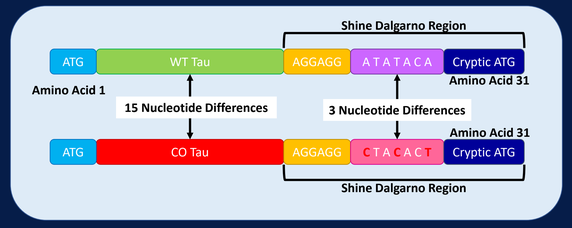

Figure 2. Comparison of Wild Type Tau and CO Tau P301L. Three differences in SD Region and 15 differences upstream.

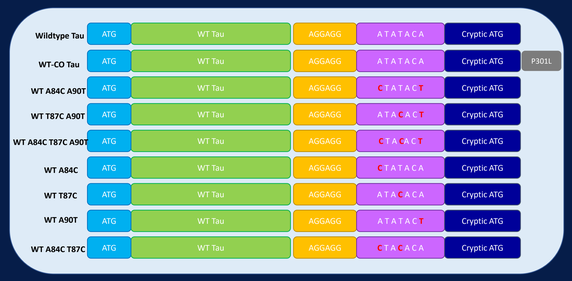

Figure 3. Constructs designed based on Wild Type Tau vector. WT CO Tau has amino acid change (Proline 301 to Leucine) to rule out the amino acid change as a cause of truncation.

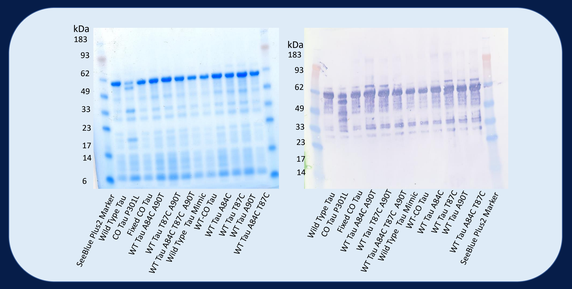

Figure 4. Gel electrophoresis with Coomassie staining and Western Blot analysis of constructs. No constructs showed truncation. Controls: Wild Type Tau, CO Tau P301, CO Fixed Tau

Strategy 2

Primer Name	Primer Description	Parental DNA
Perfect	Close match to 16s rRNA	WT Tau
Destroyed	Close match to 16s rRNA without SD sequence AGGAGG	WT Tau
Met-Val	Close match to 16s rRNA with alternative start codon Valine	WT Tau
Met-Leu	Close match to 16s rRNA with alternative start codon Leucine	WT Tau

Figure 5. Primer designs for Site Directed Mutagenesis. Developed using Agilent Primer Design Program.

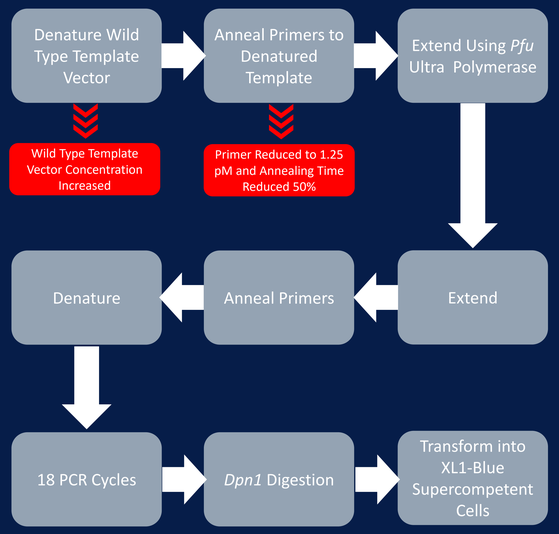

Figure 6. Site Directed Mutagenesis Schematic. Parameter changes made during process repetitions are shown in red. No successful SDM products were produced, and no colonies were yielded from transformation.

Strategy 3

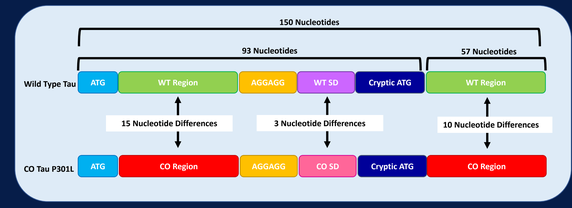

Figure 7. Comparison of first 150 nucleotides of Wild Type and CO Tau P301L. Basis of G block designs.

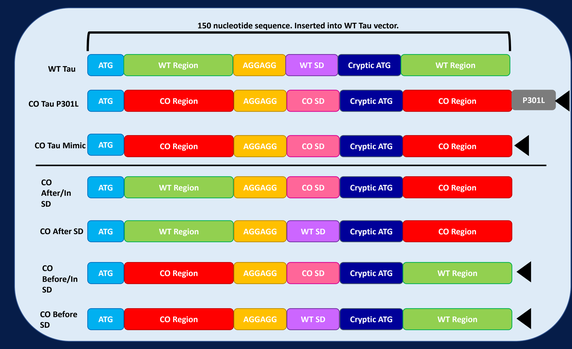

Figure 8. 150 nucleotide G block construct designs. All constructs inserted into CO Tau P301L vector by restriction enzyme digestion. Black arrows indicate truncation.

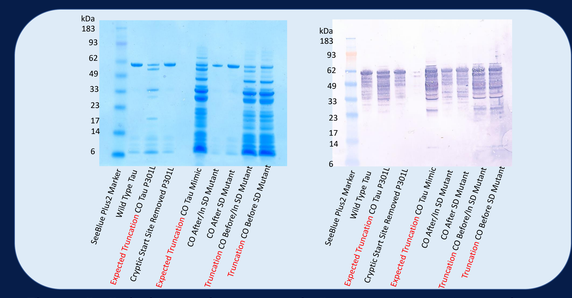

Figure 9. Gel electrophoresis with Coomassie staining and Western Blot analysis. Two designed G Block constructs within the CO Tau P301L vector showed truncation (in red).

Conclusions

- P301L mutation was not the cause of truncation in CO Tau P301L as supported by the introduction of the mutation into Wild Type Tau resulting in no truncation of expressed protein
- Nucleotide differences within the 7 base pairs between the AGGAGG Shine Dalgarno sequence and the ATG cryptic start site were not enough to induce truncation in Wild Type Tau, suggesting that sequence factors beyond the immediate SD region are at play in initiating translation downstream of the primary start site
- Truncation was apparent in G blocks that included CO Tau P301L nucleotides upstream and upstream/within the Shine Dalgarno region. This suggests that the sequence that comes before the Shine Dalgarno region effects the ability of the AGGAGG SD sequence to initiate 16s rRNA binding and initiation of translation at an alternative start site.

References & Acknowledgements

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Malys, N. (2012). Shine-Dalgarno sequence of bacteriophage T4: GAGG prevails in early genes. *Molecular Biology Reports*. 39(1): 33-39.
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