Inhibiting Amyloid Precursor Protein to Prevent Neurodegeneration in Down Syndrome and Alzheimer's Disease

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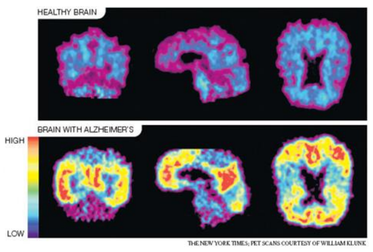
GOALS

The amyloid hypothesis suggests that reducing the amount of β-amyloid would mitigate the symptoms of Alzheimer's disease.[4] The goal of this project is to develop an aptamer for downstream therapeutic applications to inhibit amyloid precursor protein (APP) in order to alleviate conditions of Down Syndrome and Alzheimer's Disease.

BACKGROUND

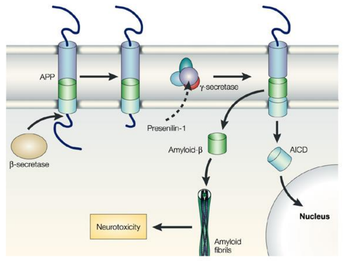
In the United States, approximately 5.2 million people have Alzheimer's Disease and more than 400,000 individuals have Down Syndrome, which is also known as trisomy 21. [1, 4] As of yet, no definitive cause or cure has been found for either condition, though evidence suggests that amyloid precursor protein (APP) and its derivative, β-amyloid, contribute to Alzheimer's disease.

Down syndrome patients have elevated APP, and therefore β-amyloid, levels since the gene for this protein is located on chromosome 21.[2] Almost everyone with Down Syndrome develops Alzheimer's due to their extra copy of APP. Approximately 5% of Alzheimer's patients have an autosomal dominant disorder that is linked to mutations in the genes for APP which lead to excessive levels of β-amyloid.[5]



PET scans of healthy and Alzheimer's brains viewed from the front, side, and top. Bright yellow and red spots show the concentration of amyloid protein, with the scale indicated to the left. *Adapted from New York Times, University of Pittsburgh Medical Center (2007).*

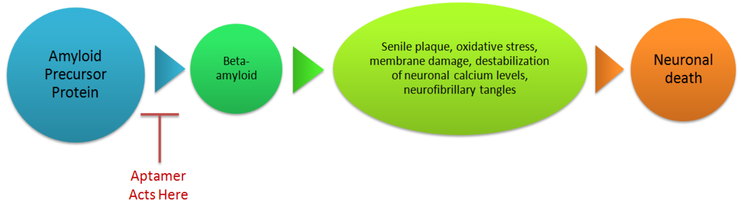
Amyloid Precursor Protein (APP) is a transmembrane integral protein concentrated in neuronal synapses. APP is cleaved by proteases into multiple peptides. One of these peptides is β-amyloid. β-amyloid peptides (amino acids 1–40 and 1–42) make up the plaques that form in the brains of Alzheimer's patients. APP intracellular domain (AICD) is another peptide derivative of APP. It is thought to regulate calcium signaling in the nucleus.[3]



Amyloid Precursor Protein Cleavage. First, β-secretase (BACE1) and then γ-secretase cleave APP to form soluble amyloid precursor protein β (sAppβ) and amyloid β42 peptide fragment. *Nature (2003).*

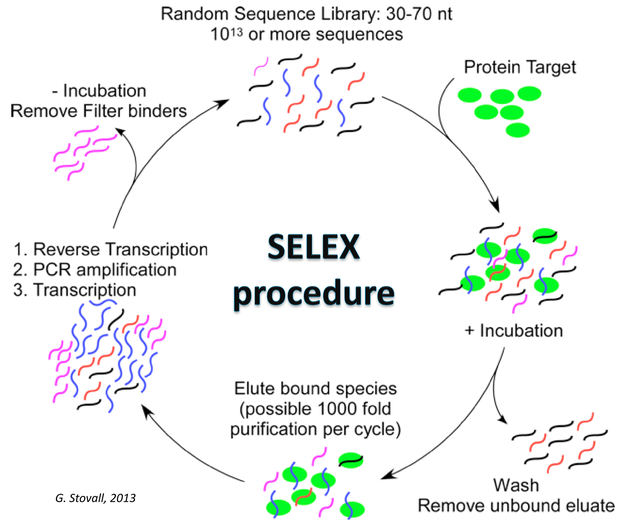
APTAMERS

Nucleic acid aptamers are a possible therapeutic method for inhibiting APP. Aptamers are small molecules of DNA or RNA selected *in vitro* from libraries of nucleic acid with random sequences of about a hundred nucleotides. Selection of a specific aptamer is based on its ability to bind the target molecule.[7]



METHODS

Systematic Evolution of Ligands by Exponential Enrichment (SELEX) is used to enrich the RNA pool in order to select for aptamers with high affinity for APP. The procedure involves bead-based selection using Histidine-tagged protein and TALON® beads, reverse transcription, PCR, transcription, and PAGE.



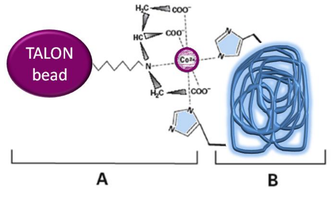
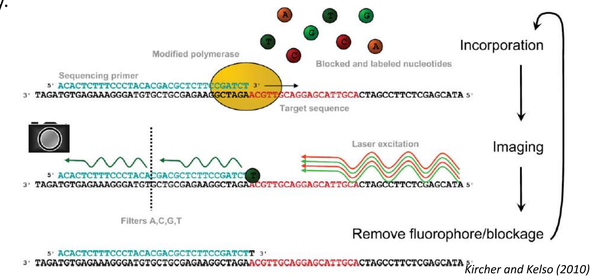


Diagram of TALON® affinity system.
- A: TALON bead – consists of a bead bearing the tetradentate chelator of the Co^{2+} metal ion.
- B: Polyhistidine-tagged protein binds to the bead through Cobalt's affinity to the amino acid Histidine

Adapted from Arizona State University, 2007

CURRENT PROGRESS

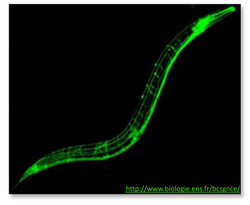
Aptamer selection has proceeded through multiple rounds and samples from round three are being sequenced using high throughput sequencing. Analyzing sequence results will allow the identification of motifs in the nucleotide sequences. These motifs will be further examined using a binding assay and Kd assay.



The Illumina Genome Analyzer uses reversible terminator chemistry for high throughput sequencing. First, special sequencing primers are annealed to copies of the DNA. Polymerases extend the sequencing primers by incorporating fluorescently labeled nucleotides that terminate replication. After the first nucleotide is added, the polymerases and free nucleotides are washed away, and the identity of the nucleotide added is determined by taking four images through different filters (T nucleotide shown here) and using two different lasers (red: A and C, green: G and T) to illuminate the fluorophores. Then, the fluorophores and terminators are removed and sequencing continues with the next nucleotide.

CONCLUSIONS AND FUTURE PLANS

Aptamers have multiple uses, diagnostic as well as therapeutic. The aptamer developed in this project will be used in experiments with *C. elegans*. *C. elegans* is a model organism that is currently being used in the Pierce-Shimomura Lab to study effects on the nervous system in Down syndrome. Using the aptamer as a detection tool as well as a treatment opens the unique opportunity to study the causes and mechanism of neurodegeneration in individuals with Down syndrome and Alzheimer's disease. Working with *C. elegans* is one step towards developing the aptamer as a therapeutic method for inhibiting APP.



C. elegans with neurons highlighted using GFP

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