Down syndrome is caused by a trisomy of chromosome 21 (having three copies instead of two copies) [1]. This extra copy of chromosome 21 is a result of abnormal cell division during development. Several neuronal abnormalities in affected individuals throughout their developmental years [2]. Down syndrome cannot be cured, and symptoms will stay with individuals for the rest of their lives. The current support and treatment can only help affected individuals manage their symptoms. Although several genes may be involved, the *DYRK1A is one* gene that has been associated with cognitive defects in Down syndrome [2]. *DYRK1A* encodes a kinase that plays a role in neural development and neuronal differentiation [3]. Interestingly, *DYRK1A* also mediates cell proliferation [4], *­and it is unclear if the levels of DYRK1A protein may mediate cell division events during neural development.*

My **primary goal** is to determine how *DYRK1A* modulates the cell cycle during neural development. I **hypothesize** that the *DYRK1A* kinase downregulates cell cycle factors leading to reduced cell proliferation during critical neural development periods. I will use wild-type and *DYRK1A* mutant zebrafish (*Danio rerio*) as a model because the *DYRK1A* domains are well-conserved, and their transparent nervous systems allow for the visualization of neural proliferation. My **long-term goal** is to analyze how neural development is impacted by cell cycle modulation.

**Aim 1: Identify the specific *DYRK1A* domains that are necessary for cell cycle processes in the brain.**

**Approach:** First, NCBI BLAST will be used to determine homologs of *DYRK1A* with diverse nervous systems. The homolog sequences will be aligned using ClustalOMEGA and highly conserved domains will be determined. I will then create *DYRK1A* mutants by using CRISPR/Cas9 to knockout the identified domains one at a time. The neural proliferation in the mutant zebrafish will be compared with the neural proliferation in the wild-type zebrafish.

**Rationale:** *DYRK1A* is known to reduce neuronal development and is also known to have phosphorylating capabilities through its P-kinase regions. Removing regions of the gene that may play a role in mediating the cell cycle in neurons is a priority as these will phenocopy the human disease.

**Hypothesis:** Knockout of the P-kinase region in species with diverse nervous systems will be important for mediating cell division in neurons.

**Aim 2: Identify genes in the brain that are expressed differently within the *DYRK1A* mutants**

**Approach:** Brain tissue samples will be taken from both wild-type and the *DYRK1A* mutant zebrafish from Aim #1. The gene expression profiles of wild-type and P-kinase knockout mutants will be determined by conducting RNA sequencing on these tissue samples. Through GO analysis, the differentially expressed genes will be sorted into categories such as neurogenesis, neural cell differentiation, and neural cell proliferation.

**Rationale:** Identifying genes that are differentially expressed in *DYRK1A* mutants will elucidate the interactions of *DYRK1A* that play a role in neural cell proliferation.

**Hypothesis:** Wild-type and *DYRK1A* mutant zebrafish will differ in gene expression profiles related to neural cell proliferation. Specifically, the *DYRK1A* mutants without the P-kinase domain will have increased expression of neural cell proliferative genes.

I will do this by isolating two groups of neural cells from the zebrafish. One group of cells will contain DYRK1A mutants without the protein kinase through crispr. The other group of cells will just be wild type and will have the p kinase domains of DYRK1A. Once isolating both groups of cells, I will conduct RNA sequencing to observe the gene expression of cell proliferative genes.

Then, I will compare gene expression profiles between the two isolated cells.

Here is a heat map of both groups of cells. Since I hypothesized that the protein kinase domain is essential for inhibiting neural proliferation. I suspect that the DYRK1A wt cells with an intact protein kinase will have decreased expression of cell proliferative genes.

The mutant cells without the protein kinase domain will show an increased expression of cell proliferative genes and will have abnormally high neural proliferation.

Approach:Visual centroposterior, medial pallium, and dorsal pallial division brain tissues samples will be taken from naïve WT and ROBO3 mutant zebrafish and from WT and mutants after completing the color maze assay multiple times. RNA-sequencing will be conducted on these tissue samples and GO analysis will sort differentially expressed genes into categories like spatial learning, visual perception, and sensory perception of light stimulus.

**Aim 3: Determine DYRK1A protein interactions that regulate the cell cycle in neurons in the brain**

**Approach**: A high-throughput chemical genomic screen will be conducted on wild-type and *DYRK1A*-overexpressed mice. I will use a focused library that consists of small molecules with a wide variety of structures known to manipulate the proteins interactions in Aim 2. The levels of cell proliferation that occurs in each treatment will be observed.

**Rationale**: Treating Ts65Dn mice with several small molecules that are known to interact with cell cycle regulators could lead to elucidating molecules that promote the functions of these proteins. Promoting cell cycle processes could rescue the reduced cell proliferation in mice with down syndrome.

**Hypothesis**: Small molecules that promote cell cycle regulator protein functions can rescue reduced cell proliferation and decrease down syndrome phenotypes in Ts65Dn mice.

Seeing the proteins that interact with *DYRK1A* and elucidating their gene ontology will allow for better understanding of what interactions are responsible for reduced cell proliferation during neuronal development.

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