**Spina Bifida,** characterized by a failure of the neural tube to fuse properly during embryonic development, is the most common birth defect and often leads to lasting disability, including paralysis and bowel dysfunction [1][2]. The **MTHFR** enzyme contributes to the re-methylation of homocysteine to produce methionine.[3] *Although phosphorylation is known to regulate this process, and heavy serine phosphorylation is predicted in the N-terminal region or MTHFR, it is not understood how this phosphorylation contributes to the development of spina bifida* [4].

The **long term goal** of this project is to understand how phosphorylation of MTHFR affects its ability to interact with the proteasome, a protein complex important for neural development and implicated in neurodegeneration. My **primary objective** for this project is to characterize the mechanism through which regulation of MTHFR by phosphorylation contributes to neural tube closure. I **hypothesize** that proper phosphorylation of MTHFR is important for neural tube closure and abnormal phosphorylation will lead to spina bifida. A zebrafish (*Danio rerio*) model will be used due to its fast development and a disease phenotype that corresponds to human spina bifida symptoms of muscle weakness, spinal defects, and bladder disfunction that are easy to see [5].

**Aim 1: Identify and mutate conserved serine phosphorylation sites.**

**Approach:** A multiple sequence alignment assay will be performed in Clustal Omega to identify conserved phosphorylation sites. CRISPR CAS9 will then be used to mutate a serine phosphorylation site in the N-terminal region of MTHFR that is conserved only in vertebrates in one population of fish (experimental mutants) and a serine conserved in all species in another population to serve as a mutant control. Wild type fish will be used as a second control.

**Hypothesis:** Mutating the phosphorylation site conserved only in vertebrates will cause a disease phenotype similar to spina bifida, characterized by a curved spine and tail and deflated swim bladder. This will not be seen in the wild type or control mutant fish.

**Rationale:** Mutating a serine phosphorylation site conserved in vertebrates will enable the identification of which structures phosphorylation affects the formation of during development. The control mutant will ensure the defects seen in the experimental mutants are specific to neurulation and not tangentially occurring as a result of a larger syndrome due to the general mutation of MTHFR phosphorylation sites.

**Aim 2: Characterize differential gene expression in phosphorylation site mutants.**

**Approach:** I will run an RNA-sequencing assay on the mutants created in aim one and compare these results to those seen in the wild type fish. I will look specifically at the genes that are differentially expressed from the wild type in the experimental mutant but not the control mutant. The biological processes affected will be categorized using gene ontology.

**Hypothesis:** I predict that several genes important for cell differentiation, migration, and division will be downregulated in the experimental mutant but not in the control mutant. Genes regarding folate metabolism will be down-regulated in both.

**Rationale:** Looking specifically at genes that are differentially expressed in the experimental mutant that are not seen in the control mutant will identify which genes that are affected by MTHFR during development are important for neural tube closure.

**Aim 3: Quantify phosphorylation during neurulation.**

**Approach:** Using mutants created in Aim 1,I will use SILAC and mass spectrometry to quantify phosphorylation. I will again look at sites that are differentially phosphorylated from wild type in the experimental but not the control mutant and categorize the affected processes using gene ontology.

**Hypothesis:** I predict that there will be significant increases in phosphorylation of proteins involved with structural development in the experimental but not the control mutants.

**Rationale:** Identifying the differences in phosphorylation sites seen in the experimental mutants will inform how phosphorylation acts to regulate biological processes affected by MTHFR to promote neural tube closure.

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