Delayed Sleep Phase Syndrome (DSPS) is a circadian rhythm sleep disorder characterized by later sleeping and waking times than conventional [1]. The Cry1 protein plays a critical component in mammals’ core circadian oscillator by regulating, through repression, the CLOCK/BMAL1 transcription/translation feedback loop [2]. Recent studies have identified a mutation in DSPS individuals with a missing segment in the Cry1 protein resulting in the lengthened circadian clock experienced by affected individuals [3]. The photolyase region of Cry1 is responsible for CLOCK/BMAL1 repression [4] and the tail region has been linked to changes in length of the circadian clock [5]. More recent studies have identified a potential phosphorylation site in the Cry1 protein tail that lengthens the circadian cycle [5] *yet the interaction of the tail region in the phosphorylation pathways of the circadian clock are still not fully understood.*

My **primary goal** is to understand the role of the Cry1 protein tail in phosphorylation and stabilization of the protein and regulation of circadian cycles. I will test the **hypothesis** that phosphorylation events in the Cry1 protein tail regulate the circadian cycle by stabilizing the Cry1 protein’s affinity to transcription factors. My **long-term goal** is to understand the processes by which the Cry1 affects circadian rhythm patterns.

**Aim #1**: Determine the conservation of amino acid domains of CRY1 and mutant sequences to determine effects of deleting the Cry1 protein tail. **Approach**: I will use CRISPR-Cas9 to create transgenic zebrafish (*Danio rerio*) lines with a deletion in the Cry1 gene segment coding for the translation of the protein’s tail. DNA samples will be sequenced from the wild-type and transgenic lines to compare the protein sequences between them. Clustal Omega will be the database used to align the sequences and demonstrate the conservation between the lines. Transgenic and WT zebrafish will be reared under equal environmental conditions and sleep-wake cycles will be monitored. **Hypothesis**: Deletions in the Cry1 gene will result in a shortening of the protein domains translated, will destabilize the protein, and will lead to the lengthening of sleep-wake cycle in the transgenic lines demonstrating the importance of this portion in the regulation of the CLOCK/BMAL1 circadian clock. **Rationale**: The demonstration of conservation of protein domains with the absence of the Cry1 protein tail will indicate the impact of this deletion when present in organisms. If the hypothesis is correct and changes to the translated protein occur, focus on the specific changes will lead to better understanding of the role of the deleted segments.

**Aim #2:** Establish the protein interactions of the transgenic zebrafish lines with the protein tail deletion. **Approach:** I will use the transgenic zebrafishlines created through CRISPR-Cas9 with a deletion in the Cry1 gene segment coding for the translation of the protein’s tail. Through tandem affinity purification, I will extract the Cry1 protein, along with proteins with which it interacts, by tagging its C-terminal. I will then characterize the captured complexes with mass spectrometry and a bioinformatic analysis. The interactions will be defined through gene ontology (GO) terms. **Hypothesis:** Mutations in the Cry1 sequence will change the protein interactions required for normal modulation of circadian clock length. **Rationale:** Determining the changes in Cry1 protein interactions with a deletion in its tail will provide insight on the role of this segment in the circadian rhythm. By looking at the changes in protein interactions, we can learn more about the mechanisms where Cry1 takes part in.

**Aim #3:** Identify chemical compounds that will interact with and potentially repress the effects of the Cry1 tail deletion. **Approach:** I will use the transgenic zebrafishlines created through CRISPR-Cas9 with a deletion in the Cry1 gene segment coding for the translation of the protein’s tail. I will then assemble a chemical library and perform a high-throughput chemical genomic screen on transgenic lines. Zebrafish will then be observed for change in activity patterns. **Hypothesis:** Small molecules that can restore the phosphorylation effects of the Cry1 protein tail and will reestablish the length of the circadian rhythm of the mutagenic lines. **Rationale:** Compounds that restore the activity patterns of the mutagenic lines will most likely function and bind similarly to the function of the missing protein tail segment. Treatments for DSPS can be designed.

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