“Evaluating the function of the Drosophila narrow abdomen ion channel using CRISPR-Cas9 genome editing”

A. Saleem¹, X. Luo², B. Lear²

¹Iowa City West High School, Iowa City, IA ²University of Iowa Department of Biology, Iowa City, IA

Background

- The narrow abdomen (na) gene in Drosophila (NALCN in humans) encodes a sodium leak channel widely expressed in the central nervous system (CNS), including circadian pacemaker neurons.
- NA/NALCN interacts with subunits UNC79 and UNC80 to form the ion channel complex (Lear et al., 2013) (refer to Figure-1). Mutations in NA/NALCN have been associated with:
  - Infanticile Neuroaxonal Dystrophy (INAD), a recessive disorder in which children suffer from motor, mental, and visual degeneration.
- Dominant mutations in humans can lead to ataxia and congenital arthrogryposis (Aoyagi et al., 2015).
- Mutations in Drosophila NA can lead to defects in circadian behavioral rhythms (Nash, 2002).

Figure 1: The NALCN (i.e. NA in Drosophila) sodium leak channel plays a pivotal role in helping neurons reach firing potential. Image from Ren et al. (2011).

Specific Goal:

Use CRISPR-Cas9 to delete a portion of the na gene and insert a donor template flanked by DNA homologous to the cut site (i.e. “Homology Arms”).

Procedure (Gratz et al., 2015)

- Design two pairs of complementary DNA sequences identical to the target sequence (homologous arms).
- Clone one of the homology arms into a pCFD3 vector (refer to Figure-4).
- The flies used for injection express a fluorescent marker, DsRed, that will make the eyes of the flies red. This is useful for gauging the success of the genetic change.
- The donor vector contains a gRNA transcription and HDR region (refer to Figure-3).
- Once the plasmids are inside the fly embryos, the U6 promoter, widely expressed in Drosophila cells, will drive the transcription of the gRNA.
- Homology arms facilitate homologous recombination of the donor template into the Drosophila genome.

Hypothesis

Missense mutations in NA/NALCN channels can have diverse effects on neuronal function and behavior depending on how the mutation affects channel regulation and/or function.

Methods

- Intro to CRISPR (refer to Figure-2)
- Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)
- Bacterial adaptive immunity
- Powerful gene-editing tool
- 2-part system
  - Cas9 cleaves the DNA
  - gRNA guides Cas9 to target sequence
- PAM binding
- Prerequisite for gRNA binding

- Three steps of CRISPR: 1) gRNA binds to Cas9; 2) gRNA binds to complementary DNA; 3) Cas9 cleaves target DNA. Retrieved from: https://www.addgene.org/crispr_guide/