Investigating the Essential Roles of dPRL-1 in Drosophila melanogaster

Alex Lee
alexlee@pugetsound.edu

Follow this and additional works at: https://soundideas.pugetsound.edu/summer_research

Part of the Biotechnology Commons, Cancer Biology Commons, Cell Biology Commons, Developmental Biology Commons, Genetics Commons, Genomics Commons, Molecular Biology Commons, and the Molecular Genetics Commons

Recommended Citation
https://soundideas.pugetsound.edu/summer_research/299

This Article is brought to you for free and open access by Sound Ideas. It has been accepted for inclusion in Summer Research by an authorized administrator of Sound Ideas. For more information, please contact soundideas@pugetsound.edu.
Investigating the Essential Roles of *dPRL-1* in *Drosophila melanogaster* (effect on embryogenesis)

Alex Lee*, Dr. Leslie Saucedo

**Abstract**

- Evidence has shown that elevated levels of PRLs are correlated with uncontrollable growth and metastasis of tumors. To investigate the normal functions of PRLs that may be exploited by cancer cells, we attempt to knock out the PRL-1 protein in fruit flies to examine its effects. We expect that PRL-1 is important for healthy embryo growth and development and so removal will lead to deleterious phenotypes. Our goal is to identify where and when PRL-1 is essential in fruit fly embryo development to gain a better understanding of how PRLs in humans contribute to developmental processes.

**Background**

- Phosphatase of Regenerating Liver (PRL) proteins regulate cell growth and division.
- Humans have three PRLs called PRL-1, PRL-2, and PRL-3 whereas *Drosophila* have only one (dPRL-1).
- Eliminating the PRL-2 gene in mice causes inhibition of growth in both embryos and adult mice.
- Knockout of *dPRL-1* will likely influence embryogenesis in a similar manner as how knockout of PRL-2 in mice affected embryogenesis; namely, growth inhibition in embryos.
- However, since *Drosophila* have only one PRL gene, PRL function can be completely knocked out unlike in mice where only one PRL gene has been knocked out at a time.

**Methods**

- Our first approach was to cause mobilization of a P element located next to *dPRL-1* in order to randomly disrupt *dPRL-1*.
- When a transposase binds to the P element, the P element is removed and genes that were near the P element often become mutated or removed as well.
- Our second approach was to use the CRISPR/Cas9 genome editing system to knock out *dPRL-1* and a yellow gene by designing a 20 nucleotide guide sequence for *dPRL-1*.
- Knocking out yellow causes flies to have yellow bodies, making it easier to screen for flies with yellow and *dPRL-1* knocked out.
- The stages of embryogenesis were stained for *dPRL-1* to determine deviations from normal expression.

**Objective(s)**

- Knock out *dPRL-1* either by random mutation (P-element approach) or directed mutation (CRISPR/Cas9 via guide RNA approach)
- Identify where and when *dPRL-1* is essential in fruit fly development

**CRISPR/Cas9-directed gene editing**

- Figure 1. CRISPR/Cas9 Gene Editing Precisely Targets Gene of Interest.
- A 20 nucleotide guide sequence binds to the Cas9 protein to form a complex. The guide RNA-Cas9 complex then binds to the PAM sequence next to the target DNA and the Cas9 protein cleaves the target DNA sequence. The cell tries to repair the double-stranded break by adding or deleting nucleotides until the two single strands become complementary. This process causes the target DNA to be excised or mutated and makes the target DNA no longer functional.

**Results (First Approach)**

- Figure 3. P-element excision. The P-element causes orange eye color; 16 lines have been established with the P-element removed (white eyes).
- Figure 4. *PRL-1* Expression in Fly Embryo. *dPRL-1* is stained in red and the cell nuclei are stained in blue.

**Results (Second Approach)**

- Figure 5. Designed 20 Nucleotide Guide Sequence for *dPRL-1* & *yellow* DNA fragments. Lane 2 contains the *dPRL-1* DNA fragment and lane 4 contains the *yellow* DNA fragment. Lanes 3 and 5 are negative controls. Both DNA fragments are approximately 300 base pairs.

**Future Directions**

- We are currently working to amplify and purify the backbone vector used to express *dPRL-1* and *yellow* guide RNAs and therefore were not successful in assembly and transformation of the completed vector.

**Acknowledgements**

- I would like to thank the University of Puget Sound Mellam Scholar Award and Dr. Leslie Saucedo for her guidance and support.

**References**

- Pagarigan et al., *PLoS One*, 2013
- Dong et al., *Biological Chemistry*, 2012