Does CRISPR gene silencing phenocopy exposure to phthalates in *Drosophila*?

a research proposal

Hailey Meagher and Kimberly Rowland, Ph.D.

LYNN UNIVERSITY
College of Arts and Sciences

Abstract

Our research group is interested in studying the effects of phthalate exposure on the *Drosophila* model. According to the literature, the Akh gene are disrupted by exposure to phthalates, which disrupts insulin-signaling (Williams et all., 2016). Our molecular biology lab recently performed CRISPR experiment (BioRad, 12012608EDU). We hypothesize that if we disrupt the same insulin signaling gene using CRISPR, we will not phenocopy the phthalate exposure results. CRISPR Cas9 is an advanced gene editing tool, that allows the detection and disruption of a target sequence in DNA. Biorad CRISPR experiment uses E. coli to demonstrate what happens when the X-gal gene is shut off, and the phenotype of the bacteria changes. We are interested in examining any differences between exposure from an epigenetic perspective and direct manipulation of genes.

Figure 1. Breakdown of CRISPR Cas9 system

Background

Exposure to phthalates in *Drosophila* disrupts the Akh gene, which are adipokinetic hormone genes, which regulate metabolism and growth in the body (Géminard et al., 2006). The disruption of the Akh gene further disturbs the insulin-signaling genes, thereby altering the resulting phenotype. Although *Drosophila* do not have pancreas, which produces insulin, they do house insulin-signaling genes, that are produced in the brain as insulin-producing cells (IPCs) that control metabolism and cell growth (Williams et al, 2016). The capabilities of CRISPR as a gene editing tool has raised further questions regarding natural epigenetic gene manipulation and the modifying of genes using a genetic tool. As we questioned from our previous understanding of CRISPR, if manipulating the same gene, Akh in *Drosophila*, will both methods conclude with similar phenocopies.

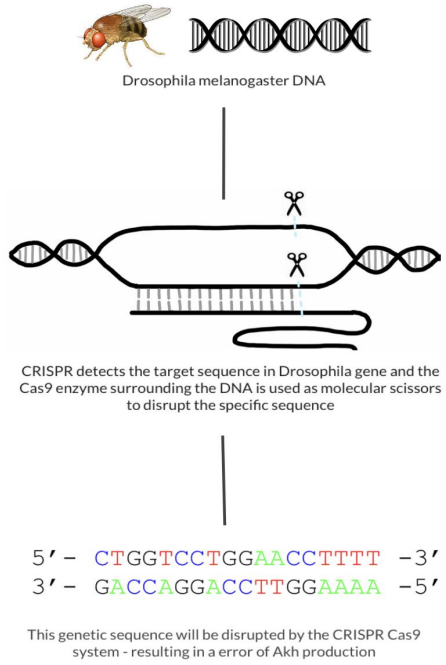
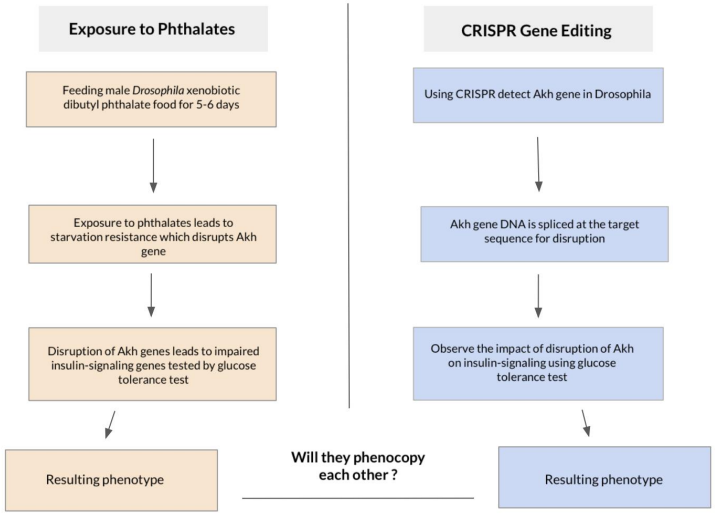


Figure 2. Schematic representation of CRISPR disruption of Akh in *Drosophila*

Figure 3. Proposed experimental methods



Predicted Results

We anticipate the CRISPR model will not phenocopy the disruption due to phthalate exposure, as we believe there are more factors involved in epigenetic disruption of insulin signaling. We anticipate a completely different phenotype when we directly target one gene. We hope to further our knowledge of CRISPR Cas9 and the differences between epigenetic and targeted-disruption phenotypes. *Drosophila* is an excellent model for understanding the interactions between genetic pathways and analysis of new techniques.

References

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Out of the Blue Crispr Kit #12012608edu https://www.bio-rad.com/en-us/product/out-blue-crispr-genotyping-extension-kits?ID=Q0JGD4E08O1Y

Williams, M. J., Wiemerslage, L., Gohel, P., Kheder, S., Kothegala, L. V., & Schiöth HB. (2016). Dibutyl phthalate exposure disrupts evolutionarily conserved insulin and glucagon-like signaling in drosophila males. *Endocrinology*, *157*(6), 2309–21. https://doi.org/10.1210/en.2015-2006