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Transcriptome Analysis of Glue Secretion in Drosophila

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TRANSCRIPTOME ANALYSIS OF GLUE SECRETION IN DROSOPHILA

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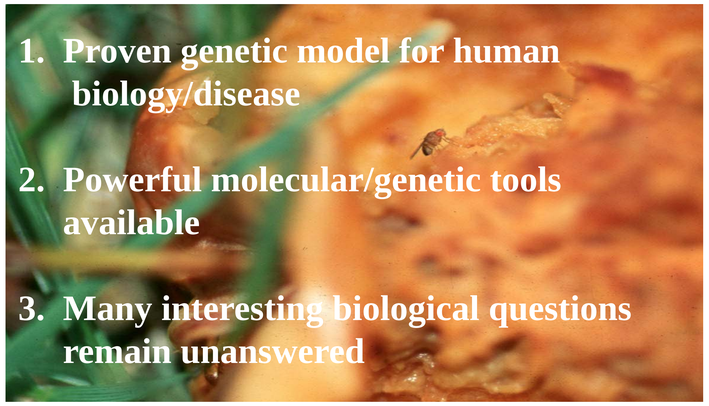
Abstract

Steroid hormones control important developmental and physiological responses in animals, including humans. It is known that when a cell is exposed to a steroid hormone, there is an immediate change in the genes that are expressed into proteins [1, 2]. Of notable importance is steroid regulation in the salivary glands of larval *Drosophila melanogaster* and the corresponding physiological responses that are governed by treatment with the conserved insect steroid, 20-hydroxyecdysone (20E). Exposure to the steroid hormone 20E causes a change in gene expression that facilitates the secretion of glue glycoproteins from inside the cells into the lumen of the tissue. Altered gene expression induced from exposure to 20E is manifested in part because of an elevation in the cytoplasmic concentration of Calcium ions (Ca^{2+}) [1, 2]. The molecular details that make the connections between the observed secretions of glue granules, the sudden increase in intracellular Ca^{2+} concentration, and the proteins that modulate these physiological responses are unknown [1], but are now experimentally tractable because of recent advances in the fields of genomics and molecular genetics. We have conducted a search through a database containing over 13,000 expressed genes from the salivary glands that show altered expression before and after hormone exposure. The goal of this research is to compile a list of candidates that show a significantly altered level of expression in preparation for functional genetic tests. Any such genes identified will be compared to human databases for shared functionality in terms of their expression and subsequent control on basic physiological responses in mammalian systems.

Introduction

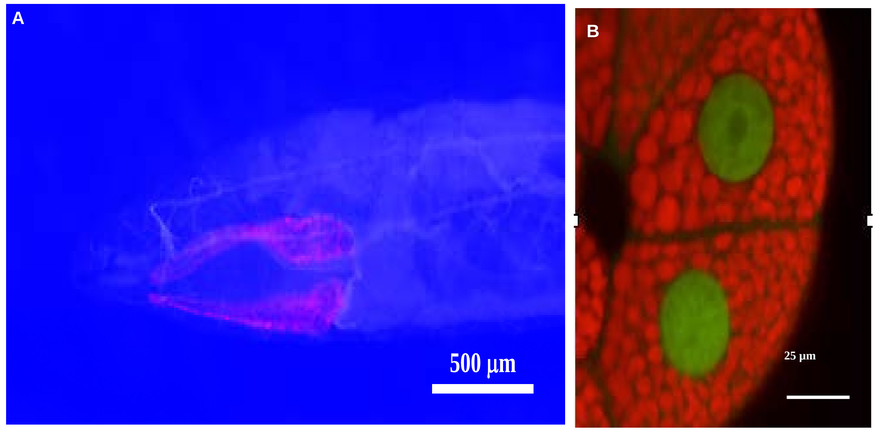
Why Flies?



Transcriptome Overview

- Genes can be viewed as constructs that contain coding information for proteins that make up a cell or organism.
- Proteins are responsible for much of the activity that occurs in the cell, but before they can be expressed, the genes responsible for them have to be transcribed into an intermediary molecule called mRNA.
- A collection of all of the mRNAs of a cell makes up a collection of the genes that are currently being used to make proteins = Transcriptome.

Why Study Salivary Glands?



1. Long history of investigating global responses to steroids (puffs on chromosomes) before the genomic era.
2. Large steroid-responsive epithelial tissue that can be cultured *ex vivo*.
3. Lots of tools to manipulate the tissue genetically.
4. Many biological responses/functions are unknown--may be a source of anti-microbial peptides.

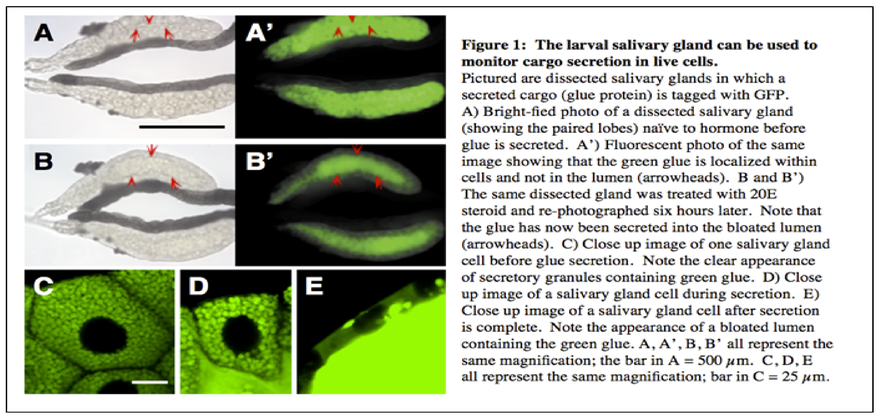
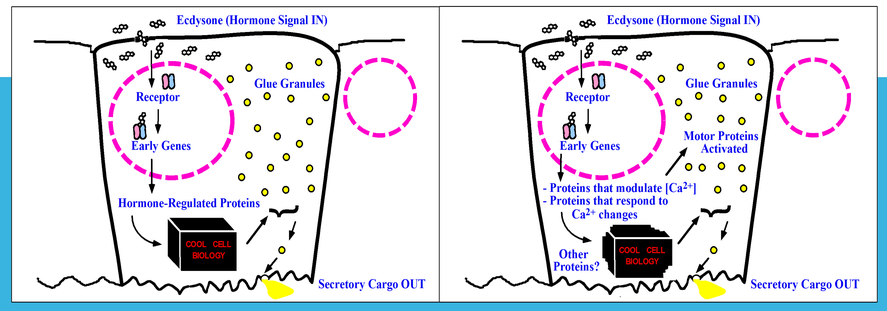


Figure 1: The larval salivary gland can be used to monitor cargo secretion in live cells. Pictured are dissected salivary glands in which a secreted cargo (glue protein) is tagged with GFP. A) Bright-fied photo of a dissected salivary gland (showing the paired lobes) naïve to hormone before glue is secreted. A') Fluorescent photo of the same image showing that the green glue is localized within cells and not in the lumen (arrowheads). B and B') The same dissected gland was treated with 20E steroid and re-photographed six hours later. Note that the glue has now been secreted into the bloated lumen (arrowheads). C) Close up image of one salivary gland cell before glue secretion. Note the clear appearance of secretory granules containing green glue. D) Close up image of a salivary gland cell during secretion. E) Close up image of a salivary gland cell after secretion is complete. Note the appearance of a bloated lumen containing the green glue. A, A', B, B' all represent the same magnification; the bar in A = 500 μm. C, D, E all represent the same magnification; bar in C = 25 μm.

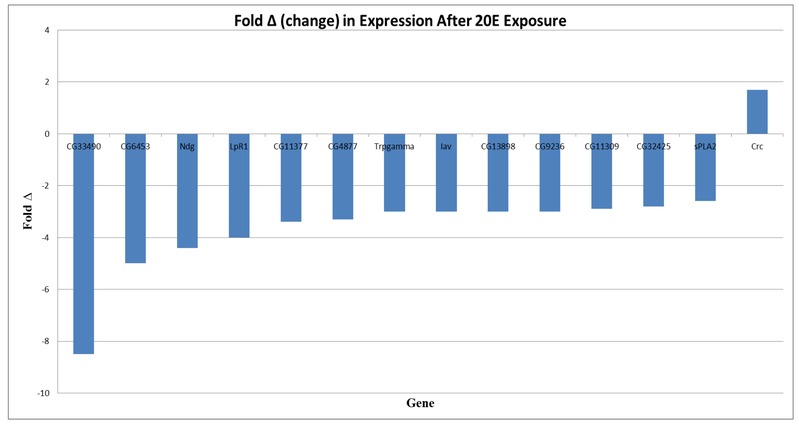
Literature Review

What We Know about Glue Secretion



- In the salivary glands of larval *D. melanogaster*, pulses of 20E leads to a transcriptional cascade that facilitates the production of glue glycoprotein and their secretion that occurs towards the end of the last larval instar (L3) [1, 2].
- The secretion of glue glycoprotein is a highly regulated process and has been shown to occur in concert with 20E secretion and Ca^{2+} changes as it has been demonstrated that at critical times in larval development, 20E is emptied into the extracellular space by means of vesicle-mediated exocytosis [2].

Results



Discussion

A transcriptome analysis identified genes that would encode proteins with domains known to be involved in Ca^{2+} signaling. Transcriptome sequencing of *D. melanogaster* generated a substantial amount of candidates for future study. Provided this list of candidates, we can now test these genes to see if they are true modulators of the steroid hormone response and examine their physiological activity.

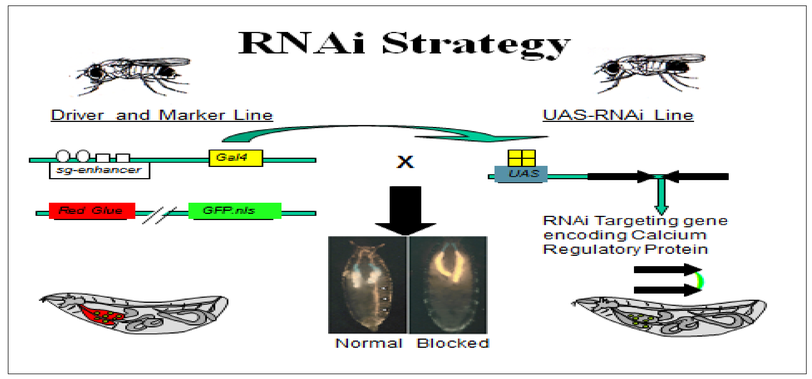


Figure 3: Strategy to test the function of genes identified from the transcriptome analysis. Stocks for each Drosophila gene that contain inverted repeats (that will trigger RNA_i) under UAS control can be obtained from one of 3 international stock centers. These will be crossed individually to a GAL4 driver expressed in the salivary gland and glue secretion can be monitored in live animals as illustrated.

Acknowledgements

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