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RNAi-mediated Knockdown of Chromatin Modifier Proteins and Their Effect on Long-term Memory in *Drosophila*

A thesis presented to Massey University in partial fulfillment of the requirements for the degree of Master of Science in Genetics

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Abstract

Memory formation in *Drosophila melanogaster* is composed of two pathways that are genetically distinct, and functionally independent of each other. These are short-term and long-term memory. Short-term memory is a transient phenomenon, located in the cytoplasm of the neuronal cells, which requires no alteration of gene expression. The formation of long-term memory requires a change in gene expression, therefore chromatin-modifying complexes may play an integral part. The mushroom-bodies of *Drosophila* are a distinct bilateral brain structure and are essential for the formation and recollection of long-term memory. Therefore, an alteration in gene expression within the mushroom bodies is essential to the formation of long-term memory. Disruption of a gene within the mushroom-bodies that resulted in an alteration in the formation of long-term memory would indicate that the gene is involved in long-term memory.

In order to investigate the role of the two chromatin-modifying proteins, HDACX and pr-Set7, whose role in memory function is unknown, RNA interference was used to knockdown expression of their respective mRNA. Published GAL4 lines were used to drive down expression in the mushroom bodies. The efficacy of the knockdown on levels of mRNA was measured by quantitative RT-PCR. The effect of these knockdowns on the formation of long-term memory was assayed using conditioned courtship. Additionally, the actual spatial and temporal expression of the GAL4 drivers was investigated using fluorescent proteins, and analysed using fluorescent microscopy.

Both pr-set7 and HDACX appear to play a role in long-term memory function. The RNAi-induced knockdown of the individual mRNAs caused impairment in long-term memory formation, although the exact mode of action is still to be elucidated. The levels of mRNA from these knockdowns were reduced within the head, although not to the extent expected. The fluorescent microscopy analysis indicated that the expression of mushroom-body specific GAL4 drivers was more widespread than previously reported.
Acknowledgment

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### Abbreviations

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<tr>
<td>BDGP</td>
<td>Berkeley <em>Drosophila</em> Genome Project</td>
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<tr>
<td>bp</td>
<td>Base Pair</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>dNTP</td>
<td>Deoxynucleoside triphosphates</td>
</tr>
<tr>
<td>CIP</td>
<td>Calf Intestinal Phosphatase</td>
</tr>
<tr>
<td>LB</td>
<td>Luria-Bertani</td>
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<tr>
<td>Amp</td>
<td>Ampicillin</td>
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<tr>
<td>PNK</td>
<td>Polynucleotide Kinase</td>
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<tr>
<td>X-gal</td>
<td>5-bromo-4-chloro-3-indolyl- beta-D-galactopyranoside</td>
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<td>CS2</td>
<td><em>chitin Synthase</em> 2 intron</td>
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<td>iPCR</td>
<td>Inverse PCR</td>
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<tr>
<td>nt</td>
<td>Nucleotide</td>
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<tr>
<td>RNAi</td>
<td>RNA Interference</td>
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<tr>
<td>HDAC</td>
<td>Histone deacetylase</td>
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<tr>
<td>HMT</td>
<td>Histone methyltransferase</td>
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<tr>
<td>HAT</td>
<td>Histone acetyltransferase</td>
</tr>
<tr>
<td>DNase</td>
<td>Deoxyribonuclease</td>
</tr>
<tr>
<td>GFP</td>
<td>Green fluorescent protein</td>
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<tr>
<td>mRNA</td>
<td>Messenger RNA</td>
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<tr>
<td>RT-PCR</td>
<td>Reverse transcriptase - polymerase chain reaction</td>
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<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
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<tr>
<td>v/v</td>
<td>Volume per volume</td>
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<tr>
<td>kb</td>
<td>Kilobase-pair</td>
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<td>CI</td>
<td>Courtship Index</td>
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<tr>
<td>MB</td>
<td>Mushroom-bodies</td>
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<tr>
<td>ISWI</td>
<td>Imitation Switch</td>
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<tr>
<td>P/CAF</td>
<td>p300/CBP Associated Factor</td>
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<tr>
<td>MYST</td>
<td><em>MOZ</em> translocation partner, two <em>yeast</em> Sas proteins, and Tip60 protein family</td>
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