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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.

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# Table of Contents

<b>Abstract.....</b>	<b>II</b>
<b>Acknowledgements.....</b>	<b>III</b>
<b>List of Table.....</b>	<b>XI</b>
<b>List of Figures.....</b>	<b>XII</b>
<b>Abbreviations.....</b>	<b>XIV</b>

<b>1. Chapter One – Introduction</b>	<b>1</b>
<b>1.1 Chromatin</b>	<b>2</b>
1.1.1 Chromatin Overview	2
1.1.2 Histone Tails	3
1.1.3 Histone Acetyltransferase	3
1.1.4 Histone Deacetylase	5
1.1.4.1 Histone Deacetylase	5
1.1.4.2 Histone Deacetylase <i>HDACX</i>	6
1.1.5 Histone Methyltransferase	7
1.1.5.1 Histone Methyltransferase	7
1.1.5.2 Histone Methyltransferase <i>pr-Set7</i>	8
1.1.5.3 Histone Demethylase	9
<b>1.2 Memory</b>	<b>10</b>
1.2.1 Memory Overview	10
1.2.2 Long-Term Memory Consolidation	11
1.2.3 Mushroom Bodies	12
1.2.3.1 Mushroom Body Development	12

1.2.3.2 Mushroom Bodies and Long-Term Memory Function	14
1.2.4 Assays for Long-Term Memory	15
1.2.4.1 Olfactory Conditioning Assay	15
1.2.4.2 Courtship Conditioning Assay	15
1.2.5 Histone Acetylation during Memory Formation	16
<b>1.3 RNAi Interference</b>	17
1.3.1 RNA interference	17
1.3.2 Molecular Mechanism of RNAi	17
<b>1.4 GAL4 Responsive Promoter</b>	19
1.4.1 Mushroom-Body Specific Drivers	19
<b>1.5 Site-Specific Integration Using Phage <math>\Phi</math>C31</b>	
<b>Intergrase</b>	20
<b>1.6 Experimental Overview</b>	21
1.6.1 Aim	21
1.6.2 Genes of Interest	21
1.6.2.1 <i>Drosophila</i> Histone Deacetylase <i>HDACX</i>	21
1.6.2.2 <i>Drosophila</i> Methyltransferase <i>pr-Set7</i>	21
1.6.3 Hypothesis	21
1.6.4 Project Objectives	22
<b>2. Chapter Two – Materials and Methods</b>	23
2.1 Chemicals and Enzymes	24
2.2 Buffers and Solutions	24
2.3 Culture Media	24
2.4 Bacterial Strains	24
2.5 Chemically Competent Cells	25
2.5.1 Modified Method for Creating Chemically Competent <i>Stbl2™</i> Cells	25
2.6 Culture Storage	25
2.7 Isolation of Nucleic Acids	25
2.7.1 RNA	25
2.7.2 Small Scale Preparation of Plasmid DNA	26

2.7.3 Large-Scale Preparations of Plasmid DNA	26
2.7.4 Isolation of Genomic DNA	26
2.7.5 Isolation and Purification of Total RNA	26
2.7.6 DNase Treatment of Total RNA	27
2.7.7 Gel Extraction	27
2.7.8 PCR Purification	27
2.8 Nucleic Acid Quantification	27
2.9 DNA Sequencing	29
2.9.1 Sequencing	29
2.9.2 Sequence Analysis	29
2.10 Polymerase Chain Reaction	30
2.10.1 PCR	30
2.10.2 PCR on Colony	30
2.10.3 cDNA Synthesis	30
2.10.4 Inverse PCR	30
2.10.5 Quantitative Real-Time PCR	32
2.11 Agarose Gel Electrophoresis	32
2.12 Enzymatic Manipulations	32
2.12.1 Analytical Restriction Digestions	32
2.12.2 Preparative Restriction Digestions for Cloning	35
2.12.3 Dephosphorylation of 5' Ends	35
2.12.4 Phosphorylation of 5' Ends	35
2.12.5 Blunted End Formation	35
2.12.6 Ligation	36
2.12.7 Transformation of DH5 $\alpha$ Chemically Competent Cells	36
2.12.7.1 Transformation of Stbl2™ Chemically Competent Cells	36
2.13 <i>Drosophila</i> Care and Manipulation	37
2.13.1 <i>Drosophila</i> Strains	37
2.13.2 <i>Drosophila</i> Culture Media	37
2.13.2.1 Cornmeal Agar	37
2.13.2.2 Egg Laying Media	37

2.13.3 Creation of transgenic Fly Lines	40
2.13.3.1 Co-Precipitation with Helper Plasmid p $\Delta$ 2-3	40
2.13.3.2 Microinjection	40
2.13.4 Microinjection Crosses	42
2.13.5 Virgin Collection	42
2.13.6 Collection of <i>Drosophila</i> Heads	42
2.13.7 Linkage Analysis	43
2.13.8 Collection of Developmental Stages	43
2.13.9 <i>Drosophila</i> Brain Dissection for Confocal Microscopy	44
2.13.10 Fluorescent Microscopy	44
2.13.11 Confocal Microscopy	44
2.13.12 Viability Assay	45
2.14 Behavioural Conditioning of <i>Drosophila</i>	45
2.14.1 Preparation of Female Flies for Conditioning Assay	45
2.14.2 Preparation of Male Flies for Conditioning Assay	45
2.14.3 Conditioning of Male Flies	46
2.14.4 Behavioural Assay Method and Materials	46
2.14.5 Statistical Analysis	48
<b>3. Chapter Three – Results</b>	<b>49</b>
3.1 Molecular Cloning	50
3.1.1 Overall Strategy for Molecular Cloning	50
3.1.2 Creation of pGEM-T Easy Clones	50
3.1.2.1 Creation of <i>HDACX</i> pGEM-T Easy Clone	51
3.1.2.2 Creation of <i>pr-Set7</i> pGEM-T Easy Clone	51
3.1.2.3 Creation of <i>msl-2</i> pGEM-T Easy Clone	51
3.1.3 Analysis of pUASp-NBa-CS2-BgX	52
3.1.3.1 Sub-Cloning of Important Regions of pUASp-NBa-CS2-BgX	52
3.1.3.2 Sequencing of Flanking Regions of the pUASp-NBa-CS2-BgX	53



3.1.4 Creation of pUASp-NBa-CS2-BgX Inverted Repeats	54
3.1.4.1 Confirmation of <i>HDACX</i> Inverted Repeats	56
3.1.4.2 Confirmation of <i>pr-Set7</i> Inverted Repeats	56
3.1.4.3 Confirmation of <i>msl-2</i> Inverted Repeats	56
3.1.5 Creation of pUASp-RNAi-attB	58
3.2 <i>Drosophila</i> Transformations	59
3.2.1 Transformation of <i>Drosophila</i> with the pUAS-IR-CS2 Vector	59
3.2.2 Transformation of pUAS-CS2-attB into <i>Drosophila</i>	59
3.2.3 Transgene Integration Sites of Selected Transformant Fly Lines	60
3.2.3.1 pUAS-HDACX <sub>IR</sub> .CS2 <sub>intron</sub> Insertion Position	60
3.2.3.2 pUAS-pr-Set7 <sub>IR</sub> .CS2 <sub>intron</sub> Insertion Position	60
3.2.3.3 pUAS-MSL2 <sub>IR</sub> .CS2 <sub>intron</sub> Insertion Position	60
3.3 Analysis of GAL4 Driver Lines by Fluorescence	61
3.3.1 Analysis of GAL4 Expression by DsRed-nls Fluorescence	61
3.3.1.1 Analysis of Artificial Promoter	61
3.3.1.2 Analysis of Enhancer-Trap Line BSC8176	65
3.3.2 Analysis of GAL4 Expression by Fluorescence of Mushroom-Body Specific Enhancers	66
3.3.2.1 Analysis of MB247 by Fluorescence	66
3.3.2.2 Analysis of MB739 by Fluorescence	70
3.3.2.3 Analysis of MB772 by Fluorescence	73
3.4 RNAi-mediated Reduction of Expression of Target Genes	75
3.4.1 Whole Fly qRT-PCR of <i>arm</i> -GAL4 Induced Transformant Lines	75
3.4.1.1 Relative Levels of <i>HDACX</i> mRNA in Whole Flies	75
3.4.1.2 Relative Levels of <i>pr-Set7</i> mRNA in Whole Flies	77
3.4.1.3 Relative Levels of <i>msl-2</i> mRNA in Whole Flies	77

3.4.2 Analysis of mRNA Levels by qRT-PCR of MB247 Induced Transformant Lines	78
3.5 <i>HDACX</i> Developmental Expression	80
3.6 Phenotypic Effect of Inverted Repeat Lines	82
3.6.1 Phenotypic Effects of Eye specific Promoter	82
3.6.2 Effect on Viability of <i>arm</i> -GAL4 Induced Transformant Lines	84
3.7 Effect of RNAi-Induced Reduction of Target Gene Expression on Long-Term Memory	86
3.7.1 Behavioural Analysis of CantonS	86
3.7.2 Behavioural Analysis of <i>CamKII</i> Inverted Repeat	88
3.7.3 Behavioural Analysis of <i>HDACX</i> Inverted Repeat	88
3.7.4 Behavioural Analysis of <i>pr-Set7</i> Inverted Repeat	91
<b>4. Chapter Four – Discussion</b>	<b>93</b>
4.1 Creation of UAS-Inverted Repeat Lines	94
4.2 Expression Analysis of GAL4 Drivers	95
4.3 Analysis of Relative mRNA Levels and Phenotypic Effects of RNAi	98
4.3.1 RNAi-mediated Knockdown of Gene Expression	98
4.3.2 Relative <i>HDACX</i> mRNA Levels Through Development	99
4.3.3 Phenotypic Effects of RNAi-mediated Knockdown of Gene Expression	99
4.4 A Possible Role for the <i>HDACX</i> and <i>pr-Set7</i> Chromatin Modifying Proteins in Long-Term Memory	100
4.5 Technical Problems Arising Within this Study	101
<b>5. Chapter Five – References</b>	<b>103</b>
<b>6. Chapter Six – Appendices</b>	<b>110</b>
6.1 Vector Map of pUAS- <i>HDACX</i> <sub>IR</sub> .CS2 <sub>intron</sub> and Predicted Restriction Fragments	111
6.2 Vector Map of pUAS- <i>prSet7</i> <sub>IR</sub> .CS2 <sub>intron</sub> and Predicted Restriction Fragments	112

6.3 Vector Map of pUAS-MSL2 <sub>IR</sub> .CS2 <sub>intron</sub> and Predicted Restriction Fragments	113
6.4 Vector Map of pUASp-NBa-CS2-BgX	114
6.5 Vector Maps of pUASp-RNAi-attB	115
6.6 Courtship Conditioning Assay Data	116
6.7 Data from qRT-PCR Experiments	117
6.8 Viability Assay Data	120
6.9 <i>Chitin Synthase 2</i> Intron Alignment	123
6.10 <i>HDACX</i> Sequence Alignment	124
6.11 <i>pr-Set7</i> Sequence Alignment	125
6.12 <i>msl-2</i> Sequence Alignment	126
6.13 Map of pCaSpeR Vector	127
6.14 Injection Data	128
6.15 Inverse PCR Sequence Alignment	129
6.16 Sequence of pUASp-NBa-CS2-BgX Region of Interest	130
6.17 Sequence of PCR to confirm <i>attB</i> insert	131

# List of Tables

Table 2.1	Oligonucleotide Primers for Sequencing and iPCR	29
Table 2.2	Oligonucleotide Primers Used for PCR	31
Table 2.3	LightCycler 480 Protocol Used for Quantitative Real-Time PCR	33
Table 2.4	Oligonucleotide Primers Used for Quantitative Real-Time PCR	34
Table 2.5	Fly Lines Used in this Study	38
Table 2.6	Fly Lines Produced in this Study	39
Table 2.7	Plasmids Used or Made in this Study	41

# List of Figures

Figure 1.1	Diagram of the <i>Drosophila</i> Mushroom-Bodies	13
Figure 1.2	Mechanism of Dicer/RISC Mediated RNAi	18
Figure 2.1	Behavioural Conditioning and Assay Chamber	47
Figure 3.1	Cloning Strategy for RNAi Vector	55
Figure 3.2	Example of Restriction Digest for Inverted Repeat Confirmation	57
Figure 3.3	GAL4 Regulated Expression of DsRed-nls Throughout Development	62
Figure 3.4	GAL4 Regulated Expression of DsRed-nls Throughout Development	64
Figure 3.5	GAL4 Regulated Expression of DsRed-nls and GFP Throughout Development	67
Figure 3.6	Confocal Microscopy Image of MB247-GAL4 Driving DsRed-nls	69
Figure 3.7	Confocal Microscopy Image of MB247-GAL4 Driving GFP	71
Figure 3.8	GAL4 Regulated Expression of DsRed-nls and GFP Throughout Development of MB739	72
Figure 3.9	GAL4 Regulated Expression of DsRed-nls and GFP Throughout Development of MB772	74
Figure 3.10	Relative mRNA Levels of RNAi Induced Transgenic Lines in Whole Flies	76
Figure 3.11	Relative mRNA Levels of RNAi Induced Transgenic Lines in Fly Heads	79
Figure 3.12	Relative mRNA Levels of <i>HDACX</i> Through <i>Drosophila</i> Development	81
Figure 3.13	Eye Specific Promoter of Inverted Repeat Lines	83
Figure 3.14	Effect of dsRNA on Fly Viability	85
Figure 3.15	Courtship Data of Adult CantonS Flies	87
Figure 3.16	Courtship Data of Adult CantonS that Express Reduced Levels of <i>CamKII</i>	89

Figure 3.17	Courtship Data of Adult CantonS that Express Reduced Levels of <i>HDACX</i>	90
Figure 3.18	Courtship Data of Adult CantonS that Express Reduced Levels of <i>pr-Set7</i>	92

# Abbreviations

BDGP	Berkeley <i>Drosophila</i> Genome Project
bp	Base Pair
BSA	Bovine serum albumin
PCR	Polymerase chain reaction
dNTP	Deoxynucleoside triphosphates
CIP	Calf Intestinal Phosphatase
LB	Luria-Bertani
Amp	Ampicillin
PNK	Polynucleotide Kinase
X-gal	5-bromo-4-chloro-3-indolyl- beta-D-galactopyranoside
CS2	<i>chitin Synthase 2</i> intron
iPCR	Inverse PCR
nt	Nucleotide
RNAi	RNA Interference
HDAC	Histone deacetylase
HMT	Histone methyltransferase
HAT	Histone acetyltransferase
DNase	Deoxyribonuclease
GFP	Green fluorescent protein
mRNA	Messenger RNA
RT-PCR	Reverse transcriptase - polymerase chain reaction
rpm	Revolutions per minute
v/v	Volume per volume
kb	Kilobase-pair
CI	Courtship Index
MB	Mushroom-bodies
ISWI	Imitation Switch
P/CAF	p300/CBP Associated Factor
MYST	<u>M</u> OZ translocation partner, two <u>y</u> east <u>S</u> as proteins, and <u>T</u> ip60 protein family