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

Charles Ellen

2008

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

I would like to thank my supervisor Associate Professor Max Scott for his seemingly endless patience, allowing me to investigate my topic freely, and reining me in if it got to out of hand. I would also like to thank him for having the confidence in me to handle a project that was new to the lab.

I would also like to thank Corey Laverty for putting up with my questions on experimental problems that had absolutely no reference to the topic at hand. Thanks to Anja for her helping me trouble-shoot the qRT-PCR experiments, and to everyone else who is in, or has been in, the Flyspot Lab: Esther, Fang, Abhi, Brandi-lee, Carolina, and Helen. I feel it is also important to mention my sister Fiona, and brother-in-law Andrew, and to thank them for the ready supply of farm meat, and handy clothes drier. The Massey University Alpine Club (MUAC) deserve a mention for simply keeping a flow of interesting people coming into the club, and helping me find places to stay around the world. Finally I would like to thank Emma for providing plenty of distraction and amusing anecdotes while sitting in the either freezing cold, or scorching hot, computer room. It was fun, and Good Luck to all.

Table of Contents

List of Tables

List of Figures

Abbreviations

BDGP	Berkeley *Drosophila* 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	*chitin Synthase 2* 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	MOZ translocation partner, two yeast Sas proteins, and Tip60 protein family