GENES AND NEURONS INVOLVED IN *DROSOPHILA* LARVAL LOCOMOTION

CHARACTERIZATION OF THE ROLE OF *dRanBPM*, *dfmr1* AND THE MUSHROOM BODY DURING LARVAL LOCOMOTION IN *DROSOPHILA*

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AIDAN DINEEN, Hon. B.Sc.

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AUTHOR: AIDAN DINEEN

SUPERVISOR: DR. ANA REGINA CAMPOS

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Abstract

Wild-type *Drosophila* larvae display photophobic behaviour when confronted with a light stimulus. This behaviour is mediated by changes in larval locomotion including increased direction change and pausing in addition to decreased contraction frequency. Foraging third instar larvae that are homozygous mutant for the *Drosophila Ran Binding Protein in the Microtubule Organizing Center (dRanBPM)* gene exhibit a reduced response to light and two alleles display a severe locomotion deficit. *dRanBPM* functional domains show a considerable number of identical amino acids when compared with orthologous genes. The human orthologue RanBPM binds to Fragile X Mental Retardation Protein (FMRP), a protein for which the loss of expression causes Fragile X syndrome. Wandering *Drosophila fragile X mental retardation 1* (*dfmr1*) mutant larvae show increased direction change and reduced time spent in linear locomotion in a dark assay. Double mutant larvae homozygous for a *dfmr1* mutant allele and carrying one copy of a *dRanBPM* mutant allele were tested for turning and response to light phenotypes. The results presented here indicate that *dRanBPM* and *dfmr1* act independently to modulate aspects of larval locomotion. Expression of dRanBPM is found in distinct sets of neurons in the larval CNS including the mushroom bodies (MBs). Neuronal silencing of the MBs in foraging third instar larvae resulted in a reduction in response to light. Finally, this reduction in response to light stimuli was characterized as a decrease in mean direction change during the light.

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List of Abbreviations

dRanBPM	*Drosophila Ran Binding Protein in the Microtubule Organizing Center*
RI	Response Index
dRanBPM	*Drosophila* Ran Binding Protein in the Microtubule Organizing Center
SPRY	Spla and the Ryanodine Receptor Domain
CTLH	C-terminal to the LisH Motif
GSC	Germline Stem Cell Niche
CNS	Central Nervous System
VNC	Ventral Nerve Cord
MBs	Mushroom Bodies
RanBPM	Ran Binding Protein in the Microtubule Organizing Center
FMRP	Fragile X Mental Retardation Protein
CRA	CT11-RanBPM Domain
TrkA	Tropomyosin-Related Kinase Receptor A
MAPK	Mitogen Activated Protein Kinase
dfmr1	*Drosophila Fragile X Mental Retardation 1*
RGG	Arginine and Glycine Rich Domain
KH	Ribonucleoprotein K Homology Domain
MAP1B	Microtubule Associated Protein 1B
KO	Knockout
mGluR	Metabotropic Glutamate Receptor
dFMRP	*Drosophila* Fragile X Mental Retardation Protein
NMJ	Neuromuscular Junction
GS	Gene-Switch
MD	Multiple Dendritic
PPK1	*Pickpocket1*
DEG/ENaC	Degenerin/Epithelial Sodium Channel Subunit
KCs	Kenyon Cells
OR	*Oregon-R*
yw	*yellow$^-$ white$^-$*
TNT-G	Active Tetanus Toxin
TNT-VIF	Inactive Tetanus Toxin
EKO	Electrically Knock Out
GFP	Green Fluorescent Protein
CSP	Cysteine String Protein
mbm^1	*Mushroom Body Miniature^1*

Chapter 1: Introduction

1.1 Photosensory System of *Drosophila*

The core neuronal circuitry underlying photosensory input in *Drosophila* larvae has been known for some time, yet the mechanism behind the modulation of larval locomotion by light has not been fully elucidated. In *Drosophila*, there are two systems that detect and convey photosensory input: the input pathways of the circadian system, which functions to entrain biological rhythms, and the visual system, which analyzes the spatial and temporal world (Helfrich-Forster et al., 2002). As in many other organisms, the adult *Drosophila* circadian system has an extraocular element. This extraretinal component designated as the Hofbauer-Buchner (H-B) eyelet consists of a cluster of Rhodopsin-6 (Rh6) expressing photoreceptor cells that project to the main circadian pacemaker neurons (Helfrich-Forster et al., 2002). These photoreceptors derive from the larval visual system structure called Bolwig's organ (Helfrich-Forster et al., 2002). Bolwig's organ is a structure of bilaterally symmetrical clusters of 12 photoreceptors with axonal projections to the optic lobe primordium (Steller et al., 1987; Campos et al., 1995; Busto et al., 1999; Hassan et al., 2000).

1.2 *Drosophila* Larval Locomotion Behaviour

Despite a spatial overlapping of the adult circadian input pathway and larval visual systems, all motor response to light in *Drosophila* larvae is regulated by the larval visual system (Hassan et al., 2005). Larval crawling behaviour is characterized by regular

muscle contractions from the posterior to anterior in a straightforward motion broken up by pauses, head swinging and turning (Berrigan and Pepin, 1995). During this movement, the head of the larva is observed to move forward in a quick motion and then towards the substrate in an effort to anchor the larvae (Berrigan and Pepin, 1995). Often, the head of the larva is unable to secure attachment and slides backwards which accounts for the noticeable shortening and lengthening of the larva (Berrigan and Pepin, 1995). In contrast to the head, the posterior of the larva consistently moves forward pausing only at the end of a contraction (Berrigan and Pepin, 1995).

The time that a larva spends in the foraging third instar stage is spent largely in the food source away from light stimulus, eating to reach a critical mass to enter pupation (Bakker, 1959). Consistent with this behaviour, larvae are repelled by a visible light stimulus (Sawin-McCormack et al., 1995). The behaviour of wild-type foraging third instar larvae was analyzed in an ON/OFF assay that subjected them to alternating pulses of light and dark (Busto et al., 1999; Scantlebury et al., 2007). This established that larvae have reduced locomotion and increased head swinging behaviour during light pulses (Busto et al., 1999). In addition to head swinging, increased direction changes result in a shorter distance traveled during the light pulse (Busto et al., 1999; Scantlebury et al., 2007). Analysis of wild-type strains revealed that larvae have significantly increased direction change going from dark to light than from light to dark (Busto et al., 1999; Scantlebury et al., 2007). Furthermore, direction change from light to dark was still higher than in complete darkness but this difference was not significant (Busto et al., 1999). All of these behaviours describe wild-type larval response to light.

Analysis of complex larval locomotion behaviour revealed that wild-type strains have a reduced frequency of muscle contractions during linear movement and reduced linear speed in the presence of light compared to dark (Scantlebury et al., 2007). This decrease in peristaltic contractions did not affect the stride length of the larvae (Scantlebury et al., 2007).

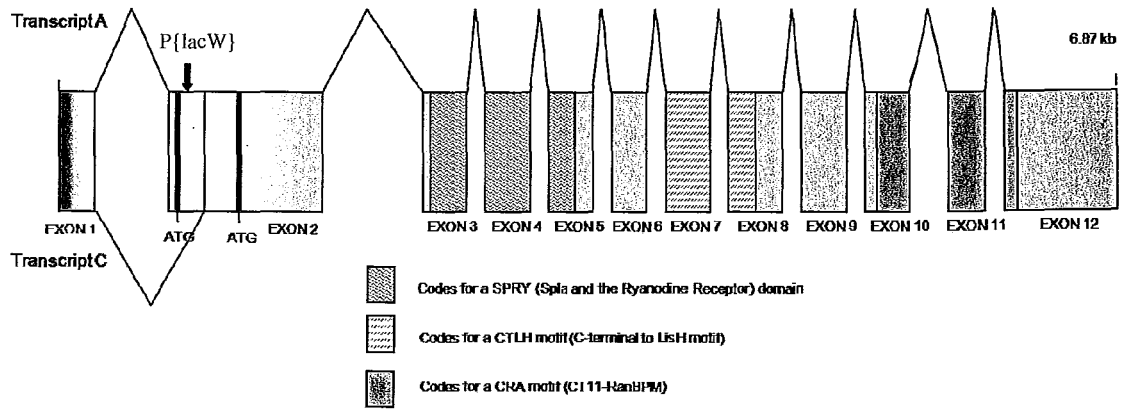
1.3 *Drosophila Ran Binding Protein in the Microtubule Organizing Center (dRanBPM)*

To better understand the underlying genetic pathway of larval response to light behaviour a mutant screen was conducted to identify genes that are required for normal response to light (Scantlebury, 2007). Candidate lines carrying an inserted P{lacW} element were screened for a reduced response to light in the ON/OFF assay as described in Scantlebury et al. (2007). A response index (RI) value was calculated for each larva as the difference of the distance the larva traveled in the light from the dark, divided by the total distance traveled in the assay. To identify mutants with a reduced response to light, mutant strains were compared with wild-type larvae to identify the strains with low mean RI values (Scantlebury, 2007). The most promising candidate gene identified in this screen was *Drosophila Ran Binding Protein in the Microtubule Organizing Center (dRanBPM* ^{k05201}*)*.

The *dRanBPM* gene is 6.87 kb in length and contains 12 exons that code for a long transcript (A) and a short transcript (C) (Fig. 1). The *dRanBPM* ^{k05201} allele carries a

Figure. 1. Organization of the *dRanBPM* gene. *dRanBPM* is composed of 12 exons and codes for two possible transcripts. The two ATG sites are shown here in exon 2. Three protein domains are depicted, the SPRY domain CTLH/LisH domain, and the CRA domain. The P{lacW} element of the *dRanBPM* ^{k05201} allele is inserted in the second exon of transcript A.

Figure. 1. Organization of the *dRanBPM* gene.



P{lacW} element inserted in the second exon, in the region that is specific to the long transcript (Fig. 1). After alternative splicing, these transcripts differ by 346 nucleotides at the 5' region of the second exon. The short transcript is predicted code for a protein of 66.4 kD protein (dRanBPM^{short}) and the long transcript is predicted to encode a 106.5 kD product (dRanBPM^{long}). The annotation of the SWISS-PROT entry of dRanBPM (accession code Q4Z8K6) lists two domains: the Spla and the Ryanodine Receptor domain (SPRY) and the C-terminal to LisH motif domain (CTLH/LisH). The SPRY domain is found in many diverse proteins and is considered to function in protein-protein interactions (Woo J.-S et al., 2006). LisH/CTLH domains are also considered to be involved in protein-protein interactions and it has been proposed that these domains can bind microtubules (Umeda et al., 2003).

The original mutant identified in the screen displayed a low RI in the ON/OFF assay and a locomotion deficit (Scantlebury, 2007). Larvae homozygous for this insertion allele displayed lethality in the third instar, decreased head swinging, reduced locomotion in the ON/OFF assay and reduced size due to lack of feeding (Scantlebury, 2007; unpublished results). Two independently created deletion mutants *dRanBPM* ^{S135} and *dRanBPM* ^{ts7} (produced by imprecise excision of P-elements), also displayed reduced RI values but in contrast to the other alleles, *dRanBPM* ^{ts7} did not exhibit a locomotion defect (Scantlebury, 2007). Mutants homozygous for *dRanBPM* ^{S135} are reduced in size and exhibit lethality in the third instar while *dRanBPM* ^{ts7} mutants die between pupation and the adult stage (Scantlebury, 2007). Larvae with heteroallelic combinations of these mutant alleles also displayed reduced response to light behaviour in the ON/OFF assay.

Furthermore, the *dRanBPM* ^{ts7} allele did not have the reduction in size phenotype found with the other two alleles. Analysis of larval feeding behaviour revealed that this decrease in larval size was due to reduced feeding of larvae that were homozygous for *dRanBPM* ^{k05201}, *dRanBPM* ^{S135} or the heteroallelic combination of these two alleles (unpublished data). Homozygous *dRanBPM* ^{ts7}larvae and larvae with heteroallelic combinations of *dRanBPM* ^{ts7} with the other two alleles did not have a feeding phenotype or a reduction in larval size (unpublished data).

The long isoform is required for maintaining the organization of the germline stem cell niche (GSC) in the ovary as its loss results in defects in cell size and leads to supernumerary GSCs attached to the niche (Dansereau and Lasko, 2008). Expression analysis in the ovary revealed a complex expression pattern as dRanBPM protein was found to be expressed in the nuclei, cytoplasm and at the cell membrane depending on the isoform, cell type and the stage during development (Dansereau and Lasko, 2008).

Further analysis of dRanBPM^{long} expression has revealed that this long isoform is expressed in the central nervous system (CNS) of foraging third instar larvae (Fig. 2, Scantlebury, 2007). Immunolabeling of the CNS using a dRanBPM^{long} specific antibody showed that the long isoform was observed to co-localize around the lateral edges of the Ventral Nerve Cord (VNC) with the neuronal marker elav (Fig.2 G-I, Scantlebury, 2007). Similar analysis of the brain lobes demonstrated that the long isoform is expressed in neurons however, expression in the lobes was stronger than in the VNC and bilaterally clustered (unpublished data). This strong bilateral expression was shown to be in the neurons of the mushroom bodies (MBs) (Fig. 2 A-E), an unmistakable and

Figure 2. dRanBPM^{long} is expressed in neurons of the mushroom bodies and the ventral nerve cord. dRanBPM^{long} co-localizes with targeted expression of UAS-CD8::GFP in the mushroom bodies using the *247*-GAL4 driver (A-F). dRanBPM^{long} co-localizes with the post-mitotic neuronal marker *elav* in the ventral nerve cord (G-I, arrow). Images in panels A-C were taken with the 20x objective, panels D-I were taken with the 63x objective. All images taken by Xiao Li Zhao.