Investigation of pathways responsible for repeat RNA-mediated cellular perturbation in *Drosophila* models of dominant expanded repeat disease

A thesis submitted for the degree of Doctor of Philosophy

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Table of Contents

Corrections

Chapter 1
Page 10, paragraph 2 should read "rather *than* enhancement"

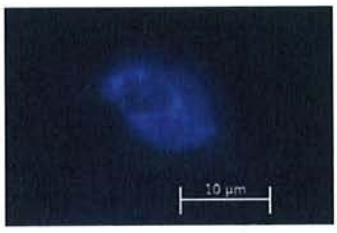
Chapter 2
Page 35, **Quantification of tergite disruption**, should include the paragraph:
The scoring scheme was based on the number and severity of disrupted tergites, using particular morphological attributes to define each category, thus minimising any experimenter bias. Preliminary data showed no significant difference (data not shown) between populations when scoring 'experimenter blind'. As such, remaining experiments were not scored blind. The order in which genotypes were scored each day was randomised and data from multiple sets of progeny obtained from multiple sets of parents on different days was used in each case.

Page 36, **Quantification of locomotion phenotype**, should include the paragraph:
Scoring involved reviewing the video to tally the time in seconds that each fly spent either upright (walking or standing) or on its back As the possibility for experimenter bias in this case appeared negligible scoring was not done 'blind'.

Page 40, **Climbing assays**, should include the clarification :
n = 3 biological replicates (sets), with 20-25 animals per genotype, per biological replicate (set), for a total of 60-75 animals examined for each genotype. A climbing score representing each biological replicate (set) was obtained by calculating the mean from 5 consecutive trials for each genotype. A final genotype score was obtained by calculating the mean of all 3 biological replicates.

Chapter 3
Page 46, **Figure 3.1** legend should include the paragraph:
Fisher's exact test does not include a calculation of standard deviation, or standard error, however 95% confidence intervals were calculated for each particular proportion. As this involved a separate calculation these values are included in Appendix 1, rather than as error bars.

Chapter 4
Page 69, In **Figure 4.1 C**, DAPI staining was poorly reproduced in the printed version. Images were chosen based on being representative of each genotype in regard to repeat RNA staining (Cy3 signal), with DAPI included as a guide to the location of the nucleus only. As such the relative levels of DAPI signal do not change the interpretation of the data. A modified version (to improve visibility in printed form) of the DAPI staining shown in 4.1 C is included below.

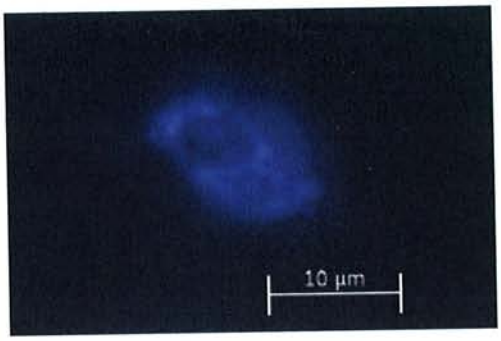


Page 77, paragraph 1, should include the paragraph:
In this study CUG-specific RNA localisation patterns were observed in independent samples from independent transgenic lines and thus the result appears robust. However, as quantification of foci was not performed, further analysis would be necessary to confirm the more subtle differences in CUG-specific localisation patterns observed in different repeat expression contexts.

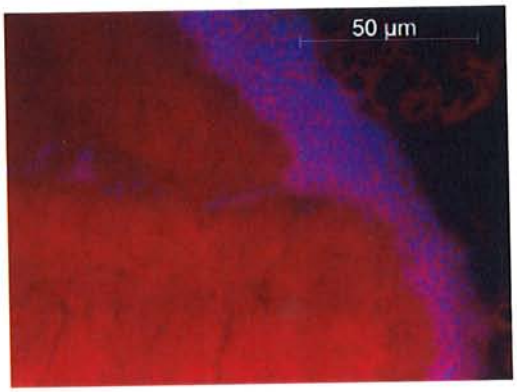
Page 80, paragraph 2, should include the sentences:
Confocal microscopy was not performed in this case. Techniques allowing higher imaging resolution may confirm the absence of neuronal foci in *Drosophila* with more certainty.

Scale bars were initially not included in fluorescent micrographs. Examples for each type of image taken are included below to aid in interpretation of these results.

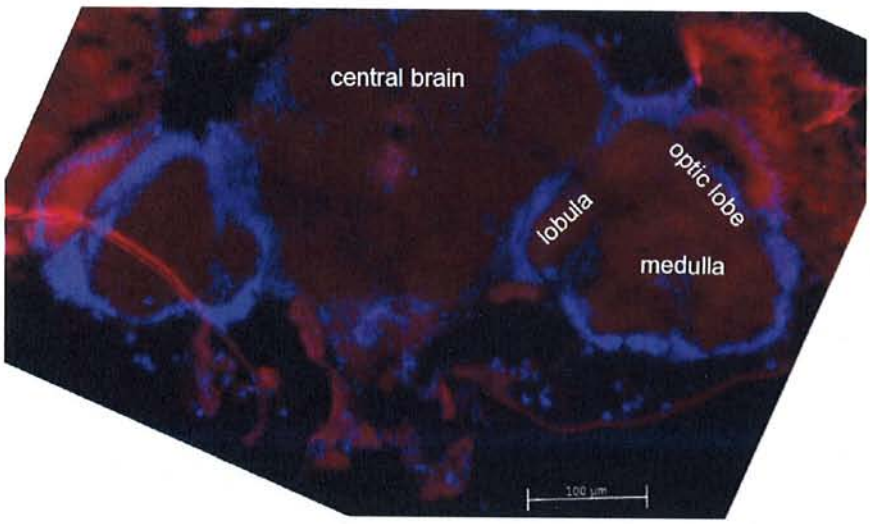
An example of muscle nuclei (As in 4.1, 4.3, 4.4, 4.5). All images were captured and cropped in the same way such that scale is identical :



An example of an adult brain at higher magnification (as in 4.6 B-D) :



An example of an adult brain at lower magnification (as in 4.6 A). In this case landmarks within the brain are annotated to further aid in interpretation.



Page 82, paragraph 1, should read :
"... support *the conclusion* that pathways ....."

Chapter 5
Page 89, paragraph 3, should read:
" ... indicate that rather *than* the insertion directly disrupting ...."

Chapter 6
Page 118, Figure 6.2 figure legend, should state:
All flies were aged for 35 days before sectioning (Materials and Methods).

Chapter 7
Page 135, paragraph 2, should read:
"In support *of* this we see alterations to miRNA profiles...."

Page 135, paragraph 3, should read:
".... indicating that, as in our model, complementary transcripts form double-stranded RNA that is processed."

Index of Figures and Tables

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Kynan Lawlor and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Kynan Thomas Lawlor

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Abbreviations

°C : degrees Celsius
% : percentage
µg : micrograms
µL : microlitre
µm : micrometre
A : adenosine
AR : androgen receptor
ATN1 : atrophin 1
ATXN1 : ataxin 1
ATXN2 : ataxin 2
ATXN3 : ataxin 3
ATXN7 : ataxin 7
ATXN8OS : ataxin 8 opposite strand
ATXN10 : ataxin 10
BAC : bacterial artificial chromosome
bp : base pairs
C : cytosine
CACNA1A : calcium channel, voltage-dependent, P/Q type, alpha 1A subunit
cDNA : complementary DNA
CLC-1 : Chloride channel 1
CNBP : CCHC-type zinc finger, nucleic acid binding protein
CUG-BP : CUG binding protein
da : daughterless
DAPI : 4'-6-diamido-2-phenylindole
DIC : differential interference contrast
DM1 : myotonic dystrophy type 1
DM2 : myotonic dystrophy type 2
DMPK : dystrophia myotonica protein kinase
DNA : deoxyribonucleic acid
dNTP : deoxyribonucleoside triphosphate
DRPLA : dentatorubral-pallidoluysian atrophy
dsRNA : double-stranded RNA
DTT : dithiothreitol
EDTA : ethylene diamine tetra-acetic acid
elav : embryonic lethal abnormal vision
ERG : electroretinogram
FMR1 : fragile X mental retardation 1
FXTAS : fragile X tremor-ataxia syndrome
G : guanosine
GFP : green fluorescent protein
GMR : Glass multimer reporter
HD : Huntington's disease
HDL-2 : Huntington's disease like 2
hnRNP : heterogenous nuclear ribonucleoprotein
HTT : huntingtin
JPH3 : juntophilin 3
kb : kilobase

KLHL1 : kelch-like 1
M : molar
mbl : muscleblind
MBNL : muscleblind-like
mg : milligrams
miRNA : micro RNA
mL : millilitres
mM : millimolar
MQ H_2O : Milli-Q (Millipore) ultrapure H_2O
mRNA : messenger RNA
mV : millivolts
ng : nanograms
PBS : phosphate buffered saline
PCR : polymerase chain reaction
pmol : picomole
polyQ : polyglutamine
polyL : polyleucine
PPP2R2B : protein phosphatase 2, regulatory subunit B, beta isoforms
RNA : ribonucleic acid
RNAi : RNA interference
rpm : revolutions per minute
RT-PCR : reverse transcription polymerase chain reaction
SBMA : spinal bulbar muscular atrophy
SCA : spinocerebellar ataxia
siRNA : small interfering RNA
SSC : saline sodium citrate
T : thymine
TAE : tris-acetate EDTA
TBE : tris-borate EDTA
TBP : TATA box binding protein
U : uracil
UAS : upstream activating sequence
UTR : untranslated region

Abstract

The expansion of polymorphic repeat sequences within unrelated genes is responsible for pathology in a family of dominant human diseases. Based on clinical and genetic similarities, it is hypothesised that common pathways may contribute to all of these diseases, with evidence for a number of mechanisms mediated by the expanded repeat. Where the repeats are translated, a long polyglutamine protein has been shown to have pathogenic properties. However, the identification of diseases caused by untranslated repeats has led to the discovery of repeat RNA-mediated pathogenic pathways. As expanded repeat-containing transcripts are present in the case of both translated and untranslated repeats, repeat RNA is a candidate common pathogenic agent. Therefore, determining its contributions to pathology will be important in understanding these diseases.

Using the model organism *Drosophila melanogaster,* this study identifies common CUG and CAG repeat RNA-mediated phenotypes, enabling the investigation of common pathways of cellular perturbation. Ubiquitous expression of either repeat sequence led to reduced viability and disruption to the development of the adult dorsal abdominal tergites through a specific effect on histoblast cells. This phenotype provides a biological read-out of common RNA-mediated effects, enabling examination of the pathways involved by quantifying the changes in the phenotype when specific candidate genes are genetically altered. Tergite disruption was not strongly modified by reducing activity of the well-characterised *muscleblind* mediated pathway. Furthermore, the presence of specific nuclear RNA foci, an indicator of repeat RNA-mediated protein sequestration, was not correlated with the phenotype. Results indicate that tergite disruption is not strongly dependent on muscleblind sequestration and may involve an alternative pathway. Ectopic expression of either repeat did not cause significant phenotypes in the eye, or neurons, except in the case of one fortuitous transgene insertion. In this case, bi-directional transcription of the repeat tract facilitated by an endogenous promoter was necessary for pathology, providing support for a novel pathway of pathology involving the formation of double-stranded RNA. Subsequent comparison of the pathways involved in hairpin-forming single stranded RNA, and bi-directional double-stranded RNA mediated phenotypes in *Drosophila* supports the existence of multiple distinct pathways that contribute to cellular perturbation.