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Co-expression of *Buffy* with *Buffy-RNAi* produces an intermediate phenotype in the *Drosophila* eye.

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Introduction

The use of ribonucleic acid interference (RNAi), a post-transcriptional gene silencing mechanism, to inhibit gene function is widely applied to analyse phenotypic consequences of gene suppression (Izant and Weintraub, 1984; Fire *et al.*, 1998; Ambesajir *et al.*, 2012). RNAi is an evolutionarily conserved cellular mechanism which is present in protozoa, fungi, nematodes, plants, flies and mammals (Agrawal *et al.*, 2003). This method is used in genome-wide screens (Dietzl *et al.*, 2007), functional genomics, genetic therapeutics, crop and animal improvements among many upcoming applications (Ambesajir *et al.*, 2012). In most of the organisms currently being used for studies, RNAi is systemic and cannot, therefore, be restricted to a specific cell type (Dietzl *et al.*, 2007). Using the bipartite UAS/Gal4 expression system (Brand and Perrimon, 1993), RNAi can be triggered in a spacio-temporal manner in *Drosophila melanogaster* (Dietzl *et al.*, 2007). Gene function can be analysed using an appropriate assay by examining the phenotypic effect of the directed inhibition (RNAi) or the overexpression of the gene. To investigate the phenotypic effects of directed overexpression upon the directed RNA interference of an important cell survival gene, we examined the consequences of the overexpression of *Buffy*, the sole pro-cell survival *Bcl-2* homologue (Quinn *et al.*, 2003), and a corresponding RNAi in the developing *Drosophila* eye. We investigated the possibility that an intermediate developmental phenotype can be generated from this interaction that may be subject to modification by other genes.

Materials and Methods

Drosophila stock and culture: *UAS-Buffy* (Quinn *et al.*, 2003) was kindly provided by Dr. Leonie Quinn (University of Melbourne). *UAS-Buffy-RNA*$_i$ (w[*]; P{w[+mC]=UAS-Buffy.RNAi}c3), *GMR-Gal4* (Freeman, 1996) and *UAS-lacZ* flies were obtained from the Bloomington Drosophila Stock Center at Indiana University. The *UAS-Buffy/CyO; GMR-GAL4* line was generated using standard recombination methods and was used to overexpress *Buffy* in the developing eye under the direction of the *GMR-Gal4* transgene. Stocks and crosses were maintained on standard medium containing cornmeal, molasses, yeast, and agar. Stocks were kept at room temperature (23^{o}C ± 2^{o}C) while crosses and experiments were carried out at 29^{o}C.

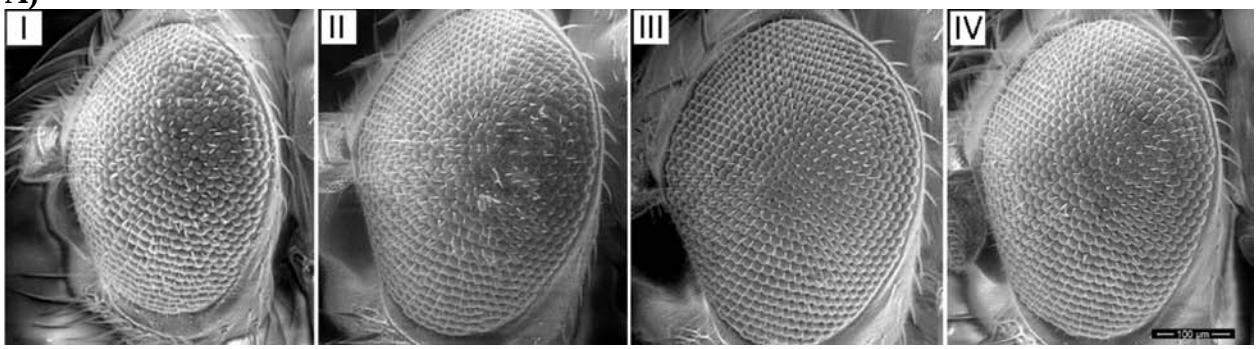
Biometric analysis of the Drosophila eye: A number of single vial crosses of each genotype were made at 29^{o}C, a cohort of the critical class male flies was collected upon eclosion and aged for three days before being frozen at -80^{o}C. Whole flies were mounted on scanning electron microscope stubs, desiccated overnight and photographed with a FEI Mineral Liberation Analyzer 650F scanning electron microscope. For each cross ten eye images were analysed using the National Institutes of Health (NIH) ImageJ software (Schneider *et al.*, 2012) and biometric analysis performed using GraphPad Prism version 5.04. The percentage

area of eye disruption was calculated as previously described (M'Angale and Staveley, 2012).

Results and Discussion

The directed expression of *Gal4* in the *Drosophila* eye at 29°C results in a roughened eye phenotype characterised by uneven, enlarged and fused ommatidia (Kramer and Staveley, 2003; Todd and Staveley, 2015). Analysis of scanning electron micrographs shows ommatidial disarray as a result of the expression of *Gal4* and the inhibition of *Buffy* in the developing eye (Figure 1A, I-II). *Gal4*-expressing flies show a disrupted ommatidia morphology, with 20% disruption of the eye (Figure 1B, I), whereas *Buffy-RNAi* flies display a much more severe phenotype of 45% disruption (Figure 1B, II). The co-expression of *Buffy* along with *Gal4* results in suppression of the roughened eye phenotype with a disruption of 3% (Figure 1B, III). The overexpression of *Buffy* along with its inhibition results in a disruption of the eye with a mean of 22% (Figure 1B, IV), intermediate between *Buffy* overexpression (3%) and its inhibition (45%). While similar to the control, this intermediate phenotype results from a balance of a rescue of the RNAi inhibition by the directed expression of *Buffy* and interference of the overexpression of *Buffy* by the RNAi transgene.

A)



B)

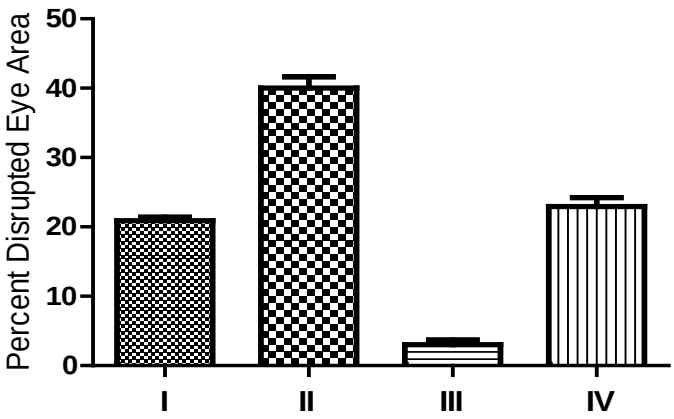


Figure 1. The phenotypic consequences of the directed expression and inhibition of *Buffy* in the Drosophila eye. A) Scanning electron micrographs of the eye of the following genotypes (I) *GMR-Gal4/ UAS-lacZ;* (II) *GMR-Gal4/ UAS-Buffy-RNAi;* (III) *GMR-Gal4 UAS-Buffy/ UAS-lacZ;* and (IV) *GMR-Gal4 UAS-Buffy/ UAS-Buffy-RNAi.* B) The biometric analysis of the eye showing the percent area of disruption (I-IV). There is suppression of the *Gal4*-expression phenotype by *Buffy* (III) and an intermediate phenotype when *Buffy* is overexpressed along with *Buffy-RNAi* (IV) as determined by one-way ANOVA and Dunnett's multiple comparison test ($P<0.05$ and 95% CI), error bars indicate the SEM and $n=10$. *(Data for GMR-Gal4/UAS-Buffy-RNAi is adapted from M'Angale and Staveley, under review.)*

Previously, our research group demonstrated the expression of the yeast transcription factor *Gal4* in the *Drosophila* eye results in apoptosis-dependent developmental defects of the ommatidial array (Kramer and Staveley, 2003). The overexpression of *Buffy*, a *Bcl-2* pro-cell survival homologue, results in the suppression of this phenotype, similar to the suppression of these developmental defects by *Pink1* (Todd and Staveley, 2015). These results suggest that the alteration of *Buffy* expression in the developing eye may subtly influence

neurogenesis. The overexpression of *Buffy* along with its inhibition results in disrupted area of the eye that is intermediate to the two extremes. Intermediate phenotypes are important in determining gene function, neuropathology of neurological diseases, and therapeutics (Civelek and Lusis, 2014; Leuchter *et al*., 2014). The inhibition of gene function by RNA interference relies on the degradation of the mRNA by the introduction of a dsRNA molecule (Boettcher and McManus, 2015). One consequence of using RNAi, for better or worse, is the generation of phenotypes that may or may not be the equivalent of null mutants

A priori, if the inhibition of *Buffy* is extremely efficient, coupled with directed overexpression of *Buffy* might be expected to generate a phenotype similar to *Buffy-RNAi* expression. Interestingly, the resulting intermediate phenotype reveals 1) that *Buffy* partially rescues the effects of *Buffy-RNAi*; 2) that *Buffy-RNAi* reduces the consequences of the directed expression of *Buffy*, and 3) that both transgenes are biologically functional. Alternatively, the overexpression of the pro-cell survival *Buffy* might be acting in a general manner to counteract the downstream effects of an overloaded "RNAi system", with the elevated levels of *Buffy* gene product being sufficient to abrogate the *Buffy-RNAi*-induced developmental eye defects. In conclusion, *GMR-Gal4* produces a cell death-dependent rough eye phenotype that can be suppressed by the pro-survival *Buffy*, enhanced by its loss of function, and the co-expression of *Buffy* along with its inhibition by RNAi results in an intermediate phenotype.

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Transferability of SRR primers developed for *D. mediopunctata* to the species *D. sturtevanti.*

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Drosophila sturtevanti (Duda, 1927) belongs to the *sturtevanti* subgroup of the *saltans* group (Magalhães, 1962). It presents a wide geographic distribution, occurring from Mexico to southern Brazil, including the Caribbean islands (Magalhães, 1962). Due to the high cost of developing microsatellite markers (SSR) for each species, the transferability of SSR primers between related species has been tested and used in population and evolutionary studies (White and Powell, 1997; Roa *et al*., 2000; Zucchi *et al*., 2002). The aim of this work was to analyze the transferability of SSR primers originally described for *D. mediopunctata* to *D. sturtevanti* (Laborda *et al*., 2009a). Twenty primers that were successfully amplified using a sample composed of a pool of individuals from an isofemale line of *D. sturtevanti*, were selected (Table 1).