Int. J. Dev. Biol. 51: 259-263 (2007)
doi: 10.1387/ijdb.062233jr

Developmental Expression Pattern

Drosophila Mrityu encodes a BTB/POZ domain-containing protein and is expressed dynamically during development

JAMIE C. RUSCONI* and UMA CHALLA

Department of Biological Sciences, University at Albany, Albany, NY, USA

ABSTRACT We report the identification and characterization of expression of a new gene in *Drosophila,* which we have named *Mrityu* (*Mri*). *Mri* was initially isolated in a microarray screen to identify molecules regulated by the transcription factor klumpfuss during retinal apoptosis. The amino acid sequence of Mri contains a BTB/POZ domain with homologues across the animal kingdom. *Mri* transcripts are present at every developmental stage as assayed by RT-PCR. We show expression of *Mri* transcripts in the female germline, confined to the nurse cells, beginning at stage 7/8. During embryonic development *Mri* is uniformly expressed early and then is refined to the gut and mesoderm primordia while expression decreases in the ectoderm. In the retina *Mri* is again expressed uniformly early, i.e., in the third instar larva and becomes more refined during pupal development where the transcripts is dynamically expressed in the cone cells and primary, secondary and tertiary pigment cells.

KEY WORDS: *Drosophila, retina, BTB/POZ domain, mesoderm, apoptosis*

Drosophila klumpfuss (*klu*) encodes a zinc finger transcription factor of the EGR-class of transcription factors (Klein and Campos-Ortega, 1997, Yang *et al.*, 1997). Members of this class of transcription factors can act as both transcriptional activators and repressors and contain a DNA binding domain of three C_2H_2 zinc-fingers (Sukhatme, 1990, Sukhatme, 1991). klu and the vertebrate Wilm's tumor suppressor-1 (WT-1) protein are unique within the EGR-class in containing four instead of three zinc-fingers (Klein and Campos-Ortega, 1997, Yang *et al.*, 1997). klu was initially characterized as a cell-fate regulator during development of the larval CNS and the adult bristles and legs (Klein and Campos-Ortega, 1997, Yang *et al.*, 1997). More recently, McDonald, et.al. identified WT-1 binding sites upstream of even-skipped (eve) and showed that these sites are regulated by klu (McDonald *et al.,* 2003). We have previously shown that *klu* is both necessary and sufficient for developmentally-regulated retinal apoptosis through regulation of dEGFR/Ras pathway activity (Rusconi *et al.*, 2004). In addition, our previous work has shown that *klu* is the first and to date only molecule identified that is differentially expressed in the interommatidial cells or secondary and tertiary pigment cells, i.e., the cells that choose to live or die, prior to activation of apoptosis in the developing retina (Rusconi *et al.*, 2004).

In an effort to identify molecules downstream of klu in apoptosis, we have recently completed a series of microarray experiments with the goal of identifying the immediate early genes that respond to klu expression (Rusconi and Cagan, unpublished). Through this work we identified a number of mRNAs whose expression levels were altered in retina overexpressing klu compared to wild-type retina.

Here, we present our characterization and expression analysis of one of these mRNAs, *Drosophila CG1216*. *CG1216* encodes a putative BTB/POZ domain containing protein with homologues across the animal kingdom. We have named this gene *Mrityu or Mri* ("death" in Sanskrit) and will call it such for the remainder of the paper. *Mri* is expressed throughout all stages of *Drosophila* development. *In situ* hybridization studies further reveal that *Mri* is expressed in the nurse cells during oogenesis and the developing mesoderm and other tissues during embryogenesis. In addition, *Mri* is dynamically expressed during retinal development, particularly during the pupal stages.

Mri (CG1216) *encodes a BTB/POZ-domain protein*

There are three predicted transcripts for *Mri* as annotated in Flybase (*Mri*-RA, -RB and -RC) (Grumbling and Strelets, 2006). Two of these transcripts *Mri*-RA and *Mri*-RB encode putative BTB/POZ-domain containing proteins. We used protein-protein BLAST and Conserved Domain Database to perform searches against all

Abbreviations used in this paper: APF, after pupa formation; BTB/POZ, broad complex tramtrack bric-a-brac/poxvirus and zinc finger; klu, klumpfuss gene; Mri, mrityu gene.

***Address correspondence to:** Dr. Jamie C. Rusconi. Department of Biological Sciences, University at Albany, Albany, NY 12222, USA. Fax: +1-518-442-4767. e-mail: jrusconi@albany.edu

0214-6282/2007/$30.00

Printed in Spain
www.intjdevbiol.com

known eukaryotic genomes to identify functional domains in the protein (Altschul *et al.*, 1990, Marchler-Bauer and Bryant, 2004). We used the multiple sequence alignment program, ClustalX and the manual sequence alignment program, SeAl, to perform alignments of vertebrate and invertebrate proteins that were identified as homologous to the putative BTB/POZ domain of CG1216/Mri (Rambaut *et al.*, 1996, Thompson *et al.*, 1997). The alignment of the BTB/POZ domains of Mri and similar proteins from other insects as well as mammals, chicken, fish, frog and worms is shown in Fig. 1 and demonstrates the conservation of this protein.

Mri *is expressed throughout* Drosophila *developmental stages*

We performed a time-course expression analysis of *Mri*-RA and –RB across the stages of *Drosophila* development using RT-PCR. We focused on *Mri*-RA and –RB because these transcripts encode the BTB/POZ domain. Using the primers described in the Experimental Procedures we show that *Mri* is expressed at all stages examined during development including in the adult female, multiple stages of embryogenesis and pupal development (Fig. 1B). These results were confirmed with an additional set of primers for a different conserved region (data not shown).

Mri *is expressed in the germline during oognesis*

We performed *in situ* hybridization to analyze the expression of *Mri-RA* and *-RB* at different stages of oogenesis using DNA probes. During oogenesis in *Drosophila* ovarioles contain temporally arranged egg chambers beginning with the germarium with the oocyte located at the posterior of each egg chamber. The remaining germ cells, the nurse cells remain toward the anterior of each egg chamber and all of these germ cells are enwrapped by a layer of somatic follicle cells (Spradling, 1993). In our studies *Mri* is absent from the germarium as well as Stages 1-6 (Fig. 2A). However, *Mri* expression is high during Stages 7-10. In these stages *Mri* is localized in the germline cells only (Fig. 2A). No expression is observed in the somatic follicle cells. Within the germline, *Mri* is concentrated in the nurse cells and appears to be absent from the oocyte (Fig. 2A). It is possible, however, that there are low levels of *Mri* in the oocyte itself that are not apparent in our experiments. It is of note that *Mri* expression is uniform in the nurse cells and does not display any transport into the oocyte even during the nurse cell dumping that occurs during stages 11 and 12 (data not shown). In addition, *Mri* was not observed in unfertilized eggs.

It is of interest to note that nurse cells undergo apoptosis shortly after "dumping" (Foley and Cooley, 1998, McCall and Steller, 1998). In addition, klu protein is highly expressed in the nurse cells (Zaffo and Rusconi, unpublished) at these same stages suggesting klu could regulate expression of *Mri* during oogenesis.

Mri *is expressed in the gut and the mesoderm and its derivatives during embryogenesis*

Mri displays a dynamic expression pattern during embryogenesis. It begins with uniform expression during the earliest stages

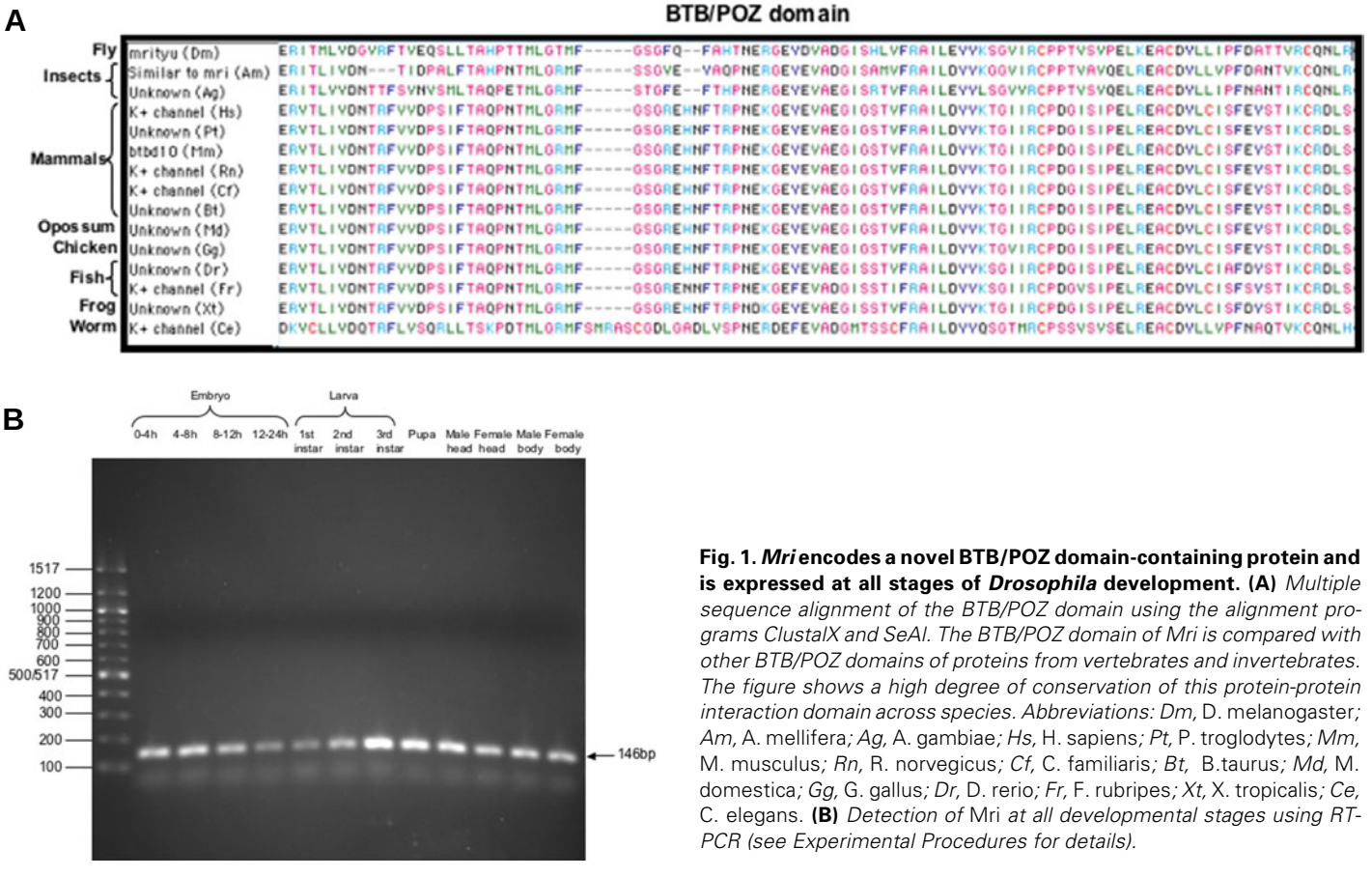

Fig. 1. *Mri* encodes a novel BTB/POZ domain-containing protein and is expressed at all stages of *Drosophila* development. (A) *Multiple sequence alignment of the BTB/POZ domain using the alignment programs ClustalX and SeAl. The BTB/POZ domain of Mri is compared with other BTB/POZ domains of proteins from vertebrates and invertebrates. The figure shows a high degree of conservation of this protein-protein interaction domain across species. Abbreviations: Dm,* D. melanogaster*; Am,* A. mellifera*; Ag,* A. gambiae*; Hs,* H. sapiens*; Pt,* P. troglodytes*; Mm,* M. musculus*; Rn,* R. norvegicus*; Cf,* C. familiaris*; Bt,* B.taurus*; Md,* M. domestica*; Gg,* G. gallus*; Dr,* D. rerio*; Fr,* F. rubripes*; Xt,* X. tropicalis*; Ce,* C. elegans. **(B)** *Detection of* Mri *at all developmental stages using RT-PCR (see Experimental Procedures for details).*

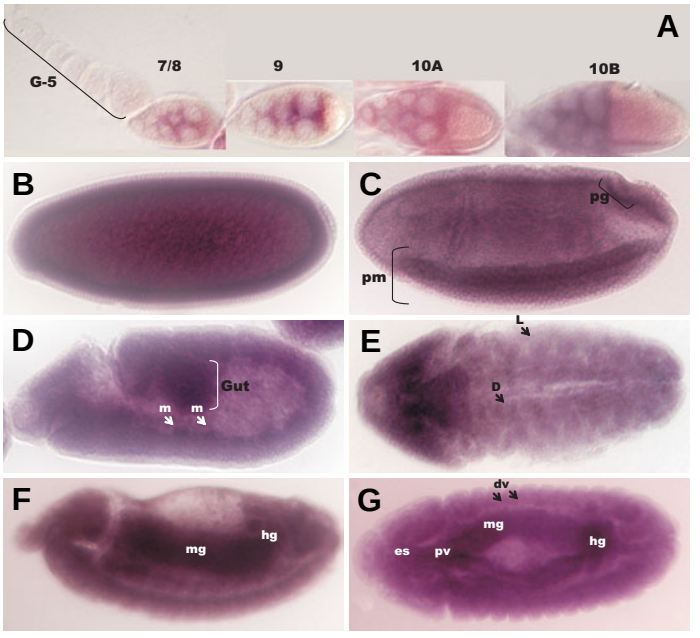

Fig. 2. Expression of *Mri* during oogenesis and embryogenesis. *No expression of* Mri *mRNA is observed prior to stage 7 of oogenesis* **(A)** *(G-5 indicates germarium through stage 5 of oogenesis). Beginning at stage 7 of oogenesis* **(A)** Mri *expression (purple) is apparent in the germ cells. More specifically,* Mri *is confined to the nurse cells and absent from the oocyte. This pattern of expression continues throughout oogenesis (9, 10A and 10B). During development of the embryo,* Mri *is expressed uniformly at early stages, e.g., cellularized blastoderm* **(B)**. *Expression of* Mri *is refined at stage 5/6* **(C)** *where expression is high in the presumptive mesoderm (pm) and gut primordia (pg). At stage 11* **(D)** Mri *is highly expressed in the invaginating gut (gut), as well as the somatic and visceral mesoderm (arrows). A filleted Stage 12 embryo in* **(E)** *shows expression of* Mri *in both the dorsal and lateral fusing somatic muscle fibers. High expression continues in the gut at later stages as viewed from both lateral and ventral views* **(F,G)** *where the midgut (mg), esophagus (es), proventriculus (pv) and hindgut (hg) are labeled. A subset of mesodermal dorsal vessel cells (dv), also express* Mri *as shown in (G) (arrows).*

of embryonic development (Fig. 2B). At Stage 5/6 of embryogenesis *Mri* is highly expressed in the presumptive mesoderm as well as in the gut primordia (Fig. 2C) and expression appears to decrease in the ectoderm. Mesodermal expression continues throughout embryogenesis. For example, at Stage 10/11 of embryogenesis *Mri* is highly expressed in the somatic and visceral mesoderm (Fig. 2D). These tissues continue to express *Mri* as is clear in developing somatic muscle fibers (Fig. 2E) and visceral (gut) musculature where the expression observed at Stage 13 is predominant in the now fully connected fore-, mid- and hind-gut (labeled in Fig. 2 F,G). *Mri* remains highly expressed in the gut at these stages (labels in Fig. 2 F and G). It is also expressed in the developing somatic mesoderm at all stages examined (Fig. 2 E,F and not shown). Due to the high levels of *Mri* expression in the visceral mesoderm and developing somatic musculature, it is difficult to ascertain if *Mri* is expressed in other mesodermal tissues, e.g., the fat body and dorsal vessel. However, expression of *Mri* is clear in a subset of dorsal vessel cells at later stages, particularly when visualized in a ventral view (data not shown and Fig. 2G).

The only apparent overlap of *Mri* and *klu* embryonic expression is the early uniform expression which could be maternal expression (Yang *et al.*, 1997). There is also no obvious overlap with regions of the embryo that undergo apoptosis, suggesting that *Mri*, like *klu*, could play a role or roles in development unrelated to apoptosis.

Mri *is dynamically expressed during retinal development*

Dynamic expression of *Mri* is also observed in the developing imaginal discs (data not shown and Fig. 3). Here, we will focus on the developing eye discs where the expression of *Mri* can be analyzed cell by cell. The most obvious landmark of development

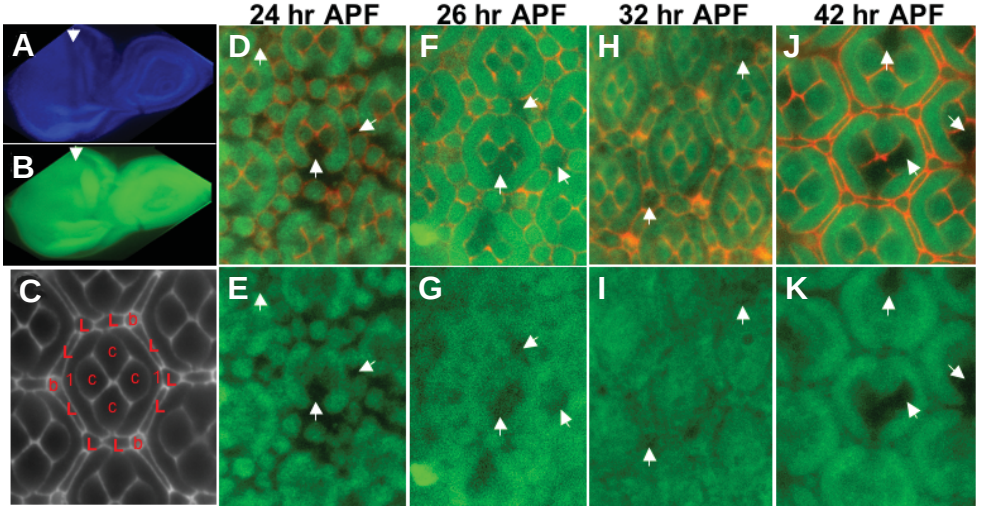

Fig. 3. *Mri* is dynamically expressed in the developing retina. Mri *mRNA (green) is uniformly expressed in the larval retina* **(B)** *both ahead of and behind the morphogenetic furrow (arrowhead).* **(A)** *The same imaginal discs as in (B) labeled with DAPI (blue) to show the nuclei and clearly visualize the morphogenetic furrow (arrowhead). There is some variable expression of* Mri *in the antennal imaginal disc (a in A,B).* **(C)** *The cell membranes of a single mature ommatidium and part of its 6 neighboring ommatidia as detected by staining of discs large protein as a reference for the remaining images.* **(D,F,H,J)** *Membranes (discs large) are in red and* Mri *mRNA is in green;* **(E,G,I,K)** *mRNA alone (green). Photoreceptors are below the surface of this apical view of the ommatidia and the other ommatidial cells are labeled as follows: cone cells (c), 1° pigment cells (1), sensory bristle groups (b) and interommatidial lattice cells or secondary and tertiary pigment cells (L).* Mri *is highly expressed in the 1° pigment cells and bristles at all time points examined. There are variations in Mri expression in both the cone cells and interommatidial cells at all time points. Arrowheads in (D-K) point to cells with little to no* Mri *expression. Intriguingly, the variable expression is not limited to particular cone cell(s) or lattice cell(s), i.e., there is no obvious pattern to the loss of* Mri *expression.*

of the larval eye imaginal disc is the movement of the morphogenetic furrow across the retina from posterior to anterior that begins the specification of retinal cell types (Heberlein, 1993, Ma, 1993). *Mri* is expressed uniformly ahead and behind as well as in the morphogenetic furrow (Fig. 3B, arrowhead marks the morphogenetic furrow). However, there are differing levels of *Mri* expression in the antennal disc (Fig. 3B). Most of the cells of the developing pupal retina are specified by 20 hours after pupa formation (APF) at 25°C. These cells include the photoreceptors (R cells), cone cells, cells of the sensory bristle groups and 1° pigment cells. The interommatidial or lattice cells, which are the precursors to the 2° and 3° pigment cells (Fig. 3C; Cagan and Ready, 1989), are not yet differentiated at this stage. Between 24 and 32 hours APF at 25°C about one third of these interommatidial or lattice cells undergo apoptosis leaving exactly six 2° pigment cells and three 3° pigment cells that along with the sensory bristle groups make up the hexagonal outline of each ommatidial. *Mri* is expressed in all R cells at all pupal stages examined (data not shown) and the same is true for the 1° pigment cells (Fig. 3 D-K). At all stages between 22 and 42 hours APF examined *Mri* is dynamically expressed in both the cone cells and the interommatidial lattice cells (not shown and Fig. 3 D-K).

Variations in *Mri* expression in the interommatidial cells is particularly intriguing as these are the cells that "choose" a life or death fate (Wolff and Ready, 1991). This makes *Mri* only the second molecule, in addition to *klu*, shown to be differentially expressed in these cells. Finally, the differential expression of both molecules implicates the possibility that klu could regulate expression of *Mri* in these cells.

Experimental Procedures

RT-PCR of developmental stages

We amplified first strand cDNA from the Rapid-Scan™ gene expression panel for *Drosophila* (Origene Technologies Inc.) using PCR with the following primers: 5'AGCACGACATAAAATCCCCTTGCGGAGCAT3' and 5'TGCTGTAAGCAGTGATTGCTCCACCGTGAA3'. The expected product is a 146bp region that is unique to *Mri-RA* and *-RB*. This experiment was repeated with primers 5'GTGATTATCTGCTCATTCCCTTCG3' and 5'CTGACACTCCTCGCCCATCT3'. With this primer set the expected product (251 base pairs) encompassed an intron to distinguish genomic DNA contamination. Expression results for this additional primer set were similar to those shown (data not shown). This gene expression panel (Origene Technologies) has been used as a semi-quantitative gene expression assay in a number of other *Drosophila* studies (Brachmann *et al.*, 2000, Hays *et al.*, 2002). In addition, a control experiment to visualize β-actin expression was performed using control primers that came with the gene expression panel. Ubiquitous β-actin expression was observed as presented in the product literature (Origene Rapid-Scan™ *Drosophila* Gene Expression Panel).

Whole mount in situ *hybridization of ovaries and embryos*

In situ hybridization of ovaries and embryos was performed as described in Rusconi and Corbin (Rusconi and Corbin, 1998). Digoxigenin-labeled DNA probes for *Mri-RA* and *-RB* were generated using standard PCR methods and the Roche PCR DIG Probe Synthesis Kit (Roche Diagnostics catalog #11636090910). Primers were designed to the same 146 base pair fragment described above for RT-PCR. After hybridization, tissue was incubated with alkaline phosphatase-coupled anti-digoxygenin antibody (Roche Diagnostics) and then the alkaline phosphatase reaction was used to visualize expression. Images were captured using DIC imaging on a Nikon Eclipse E600 with an Insight Spot digital color camera. As a control all expression was compared to background levels acquired from samples that went through the same experimental procedure with hybridization solution replacing the probe volume during the hybridization step.

In situ *hybridization and antibody staining of larval and pupal retina*

In situ hybridization of retina was performed similarly to Rusconi, Fink and Cagan (Rusconi *et al.*, 2004). The one exception being that probes were labeled with fluorescence eliminating the need for secondary antibodies to visualize localization. Alexa488 (Molecular Probes)-labeled DNA probes were generated to the 146 base pair fragment described above using standard PCR methods. After hybridization pupal retina were incubated with mouse anti-discs large antibodies (Developmental Studies Hybridoma Bank) used at a dilution of 1:10. Alex568 labeled anti-mouse antibodies (Molecular Probes) were used to visualize discs large labeling at cell membranes. Images were captured using fluorescent imaging on a Nikon Eclipse E600 with a Photometrics CoolSnapES black and white digital camera. Images were colorized and merged in Adobe Photoshop. Negative controls were performed as described above.

References

ALTSCHUL, S.F., GISH, W., MILLER, W., MYERS, E.W. and LIPMAN, D.J. (1990). Basic local alignment search tool. *J Mol Biol* 215: 403-10.

BRACHMANN, C.B., JASSIM, O.W., WACHSMUTH, B.D. and CAGAN, R.L. (2000). The Drosophila bcl-2 family member dborg-1 functions in the apoptotic response to UV-irradiation. *Curr Biol* 10: 547-50.

CAGAN, R.L. and READY, D.F. (1989). The emergence of order in the Drosophila pupal retina. *Dev Biol* 136: 346-62.

FOLEY, K. and COOLEY, L. (1998). Apoptosis in late stage Drosophila nurse cells does not require genes within the H99 deficiency. *Development* 125: 1075-82.

GRUMBLING, G. and STRELETS, V. (2006). Flybase: Anatomical data, images and queries. *Nucleic Acids Res* 34: D484-8.

HAYS, R., WICKLINE, L. and CAGAN, R. (2002). Morgue mediates apoptosis in the Drosophila melanogaster retina by promoting degradation of diap1. *Nat Cell Biol* 4: 425-31.

HEBERLEIN, U., WOLFF, T. AND RUBIN, G. M. (1993). The tgfβ homolog dpp and the segment polarity gene hedgehog are required for propagation of a morphogenetic wave in the *Drosophila* retina. *Cell* 75: 913-926.

KLEIN, T. and CAMPOS-ORTEGA, J.A. (1997). Klumpfuss, a Drosophila gene encoding a member of the EGR family of transcription factors, is involved in bristle and leg development. *Development* 124: 3123-34.

MA, C., ZHOU, Y., BEACHY, P. A. AND MOSES, K. (1993). The segment polarity gene hedgehog is required for progression of the morphogenetic furrow in the developing *Drosophila* eye. *Cell* 75: 927-938.

MARCHLER-BAUER, A. and BRYANT, S.H. (2004). Cd-search: Protein domain annotations on the fly. *Nucleic Acids Res* 32: W327-31.

MCCALL, K. and STELLER, H. (1998). Requirement for dcp-1 caspase during Drosophila oogenesis. *Science* 279: 230-4.

MCDONALD, J.A., FUJIOKA, M., ODDEN, J.P., JAYNES, J.B. and DOE, C.Q. (2003). Specification of motoneuron fate in Drosophila: Integration of positive and negative transcription factor inputs by a minimal eve enhancer. *J Neurobiol* 57: 193-203.

RAMBAUT, A., GRASSLY, N.C., NEE, S. and HARVEY, P.H. (1996). Bi-de: An application for simulating phylogenetic processes. *Comput Appl Biosci* 12: 469-71.

RUSCONI, J.C. and CORBIN, V. (1998). Evidence for a Novel notch pathway required for muscle precursor selection in Drosophila. *Mech Dev* 79: 39-50.

RUSCONI, J.C., FINK, J.L. and CAGAN, R. (2004). Klumpfuss regulates cell death in the Drosophila retina. *Mech Dev* 121: 537-46.

SPRADLING, A.C. (1993). Developmental genetics of oogenesis,. In *The Development of Drosophila melanogaster*, vol. 1 (ed. BATE, M. A. M. A., A.). Cold Spring Harbor Press, Long Island, NY., pp.1-70.

SUKHATME, V.P. (1990). Early transcriptional events in cell growth: The EGR

family. *J Am Soc Nephrol* 1: 859-66.

SUKHATME, V.P. (1991). The EGR family of nuclear signal transducers. *Am J Kidney Dis* 17: 615-8.

THOMPSON, J.D., GIBSON, T.J., PLEWNIAK, F., JEANMOUGIN, F. and HIGGINS, D.G. (1997). The Clustal_x Windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25: 4876-82.

WOLFF, T. and READY, D.F. (1991). Cell death in normal and rough eye mutants of Drosophila. *Development* 113: 825-39.

YANG, X., BAHRI, S., KLEIN, T. and CHIA, W. (1997). Klumpfuss, a putative Drosophila zinc finger transcription factor, acts to differentiate between the identities of two secondary precursor cells within one neuroblast lineage. *Genes Dev* 11: 1396-408.

Received: 25th October 2006
Reviewed by Referees: 18th December 2006
Modified by Authors and Accepted for Publication: 15th January 2007
Published Online: 27th April 2007