ScienceAsia 31 (2005): 5-11

Preliminary Studies on the Role of *ten-a* during CNS and Eye Development of *Drosophila*

Lertluk Ngernsiri,[a#*] Nora Fascetti,[b#] Wijit Wisorum[a] and Stefan Baumgartner[b]

[a] Department of Genetics, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.
[b] Section for Developmental Biology, Department of Cell and Molecular Biology, Lund University, BMC B13, Lund 22184, Sweden.

# Both authors contributed equally to the work.
* Corresponding author, E-mail: khawlln@yahoo.com

Received 23 Apr 2004
Accepted 10 Nov 2004

ABSTRACT: *ten-a* is one of the two *Drosophila* genes whose products belong to the Ten M protein family. The Ten-a protein is a type II transmembrane protein. This protein was found to be mainly expressed in the CNS of the embryo and in the compound eye of the pupa. No report about the function of *ten-a* has been published to date. Here, we showed, in a preliminary report, that *ten-a* has a function in the development of the CNS and in the compound eye. To study the function of *ten-a* during CNS development, double-stranded RNA interference (dsRNAi) experiments were performed. We found that embryos without Ten-a protein have defects in the commissures, suggesting that *ten-a* is likely to be involved in commissure formation. Furthermore, to study the function of *ten-a* in the eye, the Gal4/UAS system was used to overexpress *ten-a* at different stages of eye development. We found that extra amounts of Ten-a can interfere with the development of the eye. Flies overexpressing *ten-a* showed eye defects such as small and rough eyes, and black ommatidia. The results indicated that *ten-a* may be a novel gene involved in eye morphogenesis.

KEYWORDS: *ten-a*, *Drosophila*, compound eye, commissure, RNAi, dsRNA, Gal4/UAS system.

INTRODUCTION

The Ten M family is a new protein family that was first discovered in *Drosophila*[1] and later also in many other animals such as *C. elegans*[2], chicken[3], mouse[4], rat[5], zebrafish[3], and human[6]. Ten M proteins are about 2500–3000 amino acids in length. All members of the Ten M family are putative dimeric type II transmembrane proteins. In the type II proteins, the C-terminus is the extracellular part and, in the Ten M family, it consists of three specific domains, namely tenascin-type EGF-like repeats, a cysteine rich domain called CC-domain, and several repetitive motifs of tyrosine and aspartic acid residues, called YD-repeats. The intracellular N-terminal part contains about 150-400 amino acids[4,7]. The sequences of Ten M proteins show high homology to the extracellular matrix molecule tenascin, a major vertebrate extracellular matrix glycoprotein. Tenascins are expressed prominently in vertebrate brains throughout life[8].

Only two proteins of the Ten M family have been discovered in *Drosophila*, namely Ten-a and Ten-m[1,7,9]. The Ten-a protein is a dimeric receptor of about 500 kD. Ten-a is expressed during embryonic stages and is detected in the central nervous system (CNS), in the brain, in muscle attachment sites, in the gastric cecae, and in four spots of the antennomaxillary complex[10]. In the CNS, Ten-a is expressed only in the posterior commissures at the beginning of commissure formation, during late stage 12 and early stage 13. During later stages, Ten-a becomes expressed in the entire CNS. However, the expression of Ten-a in the posterior commissures is stronger than its expression in the anterior commissures[10]. In addition, *ten-a* transcripts were detected in the compound eyes during pupal stages[1]. The *ten-a* gene covers a genomic region of about 110 kb in length and consists of 15 exons. The gene is located on the X chromosome in the region 11A10-12 and was mapped to two sequence contigs, AE 003488 and AE 003489[10].

ten-a is one of a number of genes whose sequences are known but whose functions are still unknown. Moreover, no mutation in *ten-a* has been reported so far. To determine the function of *ten-a*, we examined the architecture of the CNS in embryos lacking the Ten-a protein. Loss of Ten-a protein was obtained by the RNA interference (RNAi) technique. We showed that loss of Ten-a by RNAi causes commissure defects. We also examined the role of *ten-a* in the compound eyes by using the Gal4/UAS system to overexpress *ten-a* in this tissue. Our observations of the eye phenotypes obtained with this system led us to suppose that Ten-a is involved in the development of the *Drosophila* compound eye.

Materials and Methods

Drosophila Strains

A wild type strain, Oregon R, was used for all dsRNAi experiments. The Gal4 lines used to drive expression specifically in the eye were *sev*-Gal4, *GMR*-Gal4, and *ey*-Gal4 from the Bloomington stock center. UAS-*ten-a 1x* and UAS-*ten-a 2x* lines were generated by cloning the cDNA of *ten-a* into pUAST. Then, the construct was introduced into the genome of w^{1118} flies by P-mediated germline transformation[11]. w^{1118} was used as wild type control in overexpression experiment.

Double-Stranded RNA Interference (dsRNAi)

dsRNAi was performed as described in Kennerdell and Carthew[12]. Briefly, the templates for synthesis of ds-*ten-a* RNA were obtained by amplifying the *ten-a* cDNA nucleotides 208-778 with the primers TGAAATCGATGAAGTACTCCG and GTTGGTGCT GTCCATGTTGGG. The 5' end of each primer was linked to the T7 promoter sequence. dsRNAs were synthesized in 50 ul of reaction solution (1 μg DNA template, 1mM of each rNTP, 10X buffer, 40 unit of T7 RNA polymerase, 20 unit of RNase inhibitor and distilled water). After incubation at 37 ^{0}C for 2 hours, the reaction was stopped by heating at 60 °C for 10 mins. The DNA templates were removed by adding 50 units RNase-free DNase. The dsRNAs were extracted with phenol/chloroform, precipitated with ethanol and dissolved in RNase-free injection buffer. The dsRNAs were injected into 0-2 hour old wild type embryos by using a microinjector. Injected embryos were allowed to develop at 18 °C until stage 16 and then they were stained with antibodies. Injection with buffer alone was used as a negative control.

Antibody Staining

Primary antibodies used for staining the CNS were anti-Ten-a (1:50), mAb BP102 (1:200) and mAb Fasciclin II (1:5). The secondary antibodies were anti-mouse IgG and anti-rabbit IgG conjugated to horseradish peroxidase (Sigma). The staining procedure was modified from Patel[13]. Briefly, stage 16 injected embryos were washed with PBS + 0.01% Triton-X to remove the injection oil. Then, embryos were removed from coverslides and fixed in 4% formaldehyde for 30 mins. The embryos were devitellinised by hand and incubated overnight with the primary antibody. Then, the embryos were washed to remove the excess antibody and incubated with the secondary antibody for 3 hours. After washing with PBS to remove the excess secondary antibody, the embryos were colored by adding 1 ml of DAB solution (750 μl of 0.3% diaminobenzidine, 250 μl PBS, 2 μl of 0.3% hydrogen peroxide), the color was monitored and the reaction was stopped by adding PBS.

Overexpression Experiments

The Gal4/UAS system[14] was used to overexpress *ten-a* in the compound eye. Experiments were carried out by crossing each driver line to UAS-*ten-a 1x*, UAS-*ten-a 2x* and w^{1118}. The progeny were reared at 25°C. In each cross, the compound eyes of adult progeny were observed, and photographs were taken by an Olympus stereomicroscope.

Results

Ten A Protein is Inhibited by ds-*ten-a* RNA

Ten-a protein has been reported to be mainly expressed in the CNS of *Drosophila* embryos[10]. We anticipated that *ten-a* might play a role in the development of the CNS. In order to define the function of *ten-a* during the development of the CNS, we performed double-stranded RNAi experiments. The dsRNAi method has been proven to be very powerful to study gene function in many organisms such as *C. elegans*[15], *Drosophila*[12], *Trypanosoma*[16], mouse embryos[17], and *Arabidopsis*[18]. In this method, the target gene is still expressed but its transcript is degraded by dsRNA with corresponding sequence. Due to the absence of the mRNA, the protein product of the target gene can not be synthesized[19]. Since the absence of the protein product is equivalent to disrupting the gene function, the phenotype induced by dsRNAi is similar to that obtained with loss-of-function mutations.

We generated 624 bp long ds-*ten-a* RNAs *in vitro*. The sequence of ds-*ten-a* RNAs corresponded to the coding sequence immediately after the first methionine. The ds-*ten-a* RNAs were injected into wild type embryos and the embryos were allowed to develop until stage 16 before staining them with anti-Ten-a antibody against Ten-a protein. In wild-type stage 16, embryos, anti-Ten-a stained prominently the entire CNS[1,10]. We found that ds-*ten-a* RNA injected embryos did not stain with the anti-Ten-a (data not shown). By contrast, the CNS of buffer injected embryos showed an identical staining pattern to that of wild type embryos (Fig. 1A). The ds-*ten-a* RNA injected embryos and the buffer injected embryos were also stained with monoclonal antibody BP102 (Fig. 2), a specific antibody staining all axons of the *Drosophila* embryonic CNS[20].

The result of our dsRNAi experiments showed that Ten-a protein could not be produced in embryos injected with ds-*ten-a* RNA. If the Ten-a protein could be produced, the embryos would show staining with the anti-Ten-a, whereas embryos lacking Ten-a protein would be negative to the staining. The CNS of ds-*ten-a* RNA injected embryos could be stained with mAb BP102 because the protein substrate of this antibody was not

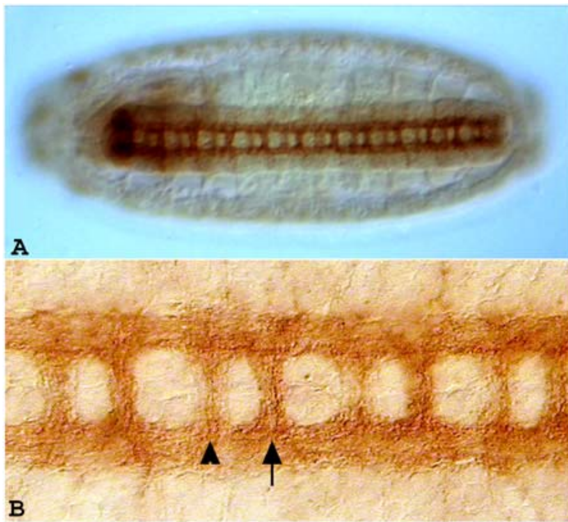

Fig 1. The wild-type CNS pattern revealed by staining with anti-Ten-*a*. (A) The wild type CNS pattern is revealed by anti-Ten-a. (B) Higher magnification view. Arrowhead points to the anterior commissures and arrow points to the posterior commissures.

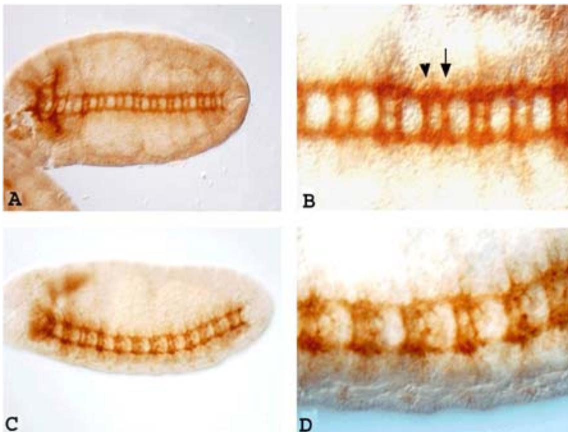

Fig 2. Defects of CNS pattern caused by ds-*ten-a* RNA revealed by mAb BP102. (A and B) The CNS architecture of a buffer-injected embryo stained. (C and D) The CNS architecture of embryos injected with ds-*ten-a* RNA. Arrowhead points to the anterior commissures and arrow points to the posterior commissures.

knocked-down. Therefore, the ds-*ten-a* RNA specifically degraded *ten-a* mRNA and only the Ten-a protein was knocked-down.

Loss of Function of *ten-a* Causes Defects in Commissures

The primary aim of this work was to examine the defects in the CNS when *ten-a* function was disrupted. The *Drosophila* CNS has a ladder-like structure. Two longitudinal connectives run on each side of the midline along the antero-posterior axis of the embryo. Each segment has two commissures, namely the anterior and posterior commissures, connecting the two longitudinal connectives. Each commissure consists of a bundle of axons crossing the midline to the opposite side [21].

In the ds-*ten-a* RNA injected embryos, staining with monoclonal antibody BP102 revealed obvious defects in the commissures, particularly in the posterior commissures. Such commissures were fuzzy, partially fused or completely fused (Fig.2 C,D). The defective commissures were present in 63% of all scored segments. Moreover, we observed that in each embryo, the number of affected segments ranged between five to nine segments (Table 1). Commissures of control embryos injected with buffer had a wild-type appearance (Fig. 2 A,B). The posterior commissures were more disrupted then the anterior commissures. Often posterior commissures where thinner than normal and, in the most extreme cases, they did not form. It has been reported that during early stages of embryonic CNS development, Ten-a protein is mainly expressed on the axons extending across the midline via the posterior commissures[10]. The commissural defects caused by ds-*ten-a* RNAi might have been due to the fact that axons crossing the posterior commissure lacked Ten-a protein to guide them properly and to allow them to form a bundle. These axons might be unable to distinguish their route and cross the midline in a disorganized fashion. Therefore, we would like to suggest that Ten-a might be a labeling molecule necessary for axons to distinguish between the anterior and posterior commissures during the beginning of their formation (see Discussion).

Table 1. The number of detective commissures induced by ds-*ten-a* RNA.

	Number of counted segments	Number of detective larvae					%
		Number of segments 5	6	7	8	9	
ds RNA	456	2	7	14	13	2	63.5
Buffer	420	0	0	0	0	0	0

Some Injected Larvae could not Move

In addition to the phenotypes mentioned above, we also observed that some embryos injected with ds-*ten-a* RNA had defective muscles or motor control. We found that 40% of the injected embryos that were allowed to develop to larval stage could not move or did not move enough to hatch from the vitelline membrane (Table 2). As described above, Ten-a is also expressed in muscle attachments and ds-*ten-a* RNA can completely inhibit Ten-a production. The absence of Ten-a in muscle attachments may be the cause of larval immobilization. Moreover, aberrant crossing of the midline might cause motorneurons to project erroneously to muscles. In the wild-type embryo, each motorneuron exits the CNS and innervates a specific muscle of the body wall. To investigate this, we stained the injected embryos with a monoclonal antibody against Fasciclin II, a specific antibody for motorneuron projections[22]. However, the Fasciclin II staining pattern of ds-*ten-a* RNA injected embryos was indistinguishable from that of wild-type embryos (data not shown). Therefore, it is likely that immobile larvae resulted from the loss of Ten-a in muscles attachments. However, since 60% of dsRNA injected embryos that fully developed could hatch from the vitelline membrane, and some of the unhatched larvae could slightly move, the level of immobilization might vary according to the number of muscles that fail to attach.

ten-a is Involved in the Development of the Compound Eyes

In addition to expression in the CNS, *ten-a* expression has also been reported in the eye of pupae[1]. We therefore presumed that *ten-a* might have a role during eye development. To study the role of *ten-a* during eye development, we employed the Gal4/UAS system. With this system it is possible to overexpress *ten-a* only in the developing eye[14]. To do this, we cloned a full-length *ten-a* cDNA into the pUAST vector and established transgenic lines using germline transformation. Two UAS-*ten-a* lines were generated: UAS-*ten-a 1x* carrying one copy of the *ten-a* transgene, and UAS-*ten-a 2x* harboring two copies of the transgene. The line w^{1118}, which had no transgene, was used as a negative control line. The Gal4 driver lines used in this experiment were *sev*-Gal4, GMR-Gal4 and *ey*-Gal4. Each driver line was crossed to both UAS lines and to the w^{1118} line. Then, the compound eyes of the progeny were examined. We found that these compound eyes showed different defects depending on the driver line, whereas no defects were found in the compound eyes of the progeny from the crosses between all drivers and w^{1118} (Fig. 3A, D, G).

The *sev*-Gal4 line uses the *sevenless* promoter to drive expression of Gal4 in differentiating photoreceptors (R3, R4, R1, R6 and R7) and cone cells of the eye discs[23,24]. The progeny from *sev*-Gal4 X w^{1118} showed normal eyes. Conversely, ectopic expression of *ten-a* induced by *sev*-Gal4 caused the presence of black dots in the eyes. The progeny from *sev*-Gal4 x UAS-*ten-a 1x* showed a slightly lower number of black dots than that from *sev*-Gal4 x UAS-*ten-a 2x* (Fig 3B,C). These black dots indicated death of ommatidia[25]. Other features of the eye, such as the size and composition of the ommatidia, were relatively normal. The compound eye is derived from an epithelium monolayer called an eye imaginal disc that, during the third larval stage, begins differentiating into an eye specific tissue. The cells of the eye disc epithelium differentiate as a wave, called the morphogenetic furrow, which sweeps across the disc from the posterior margin to the anterior end of the disc. Anterior to the morphogenetic furrow, cells still proliferate actively and do not differentiate. In the morphogenetic furrow cells stop proliferating and begin to differentiate. Posterior to the furrow, all cellular components are recruited and assembled into the ommatidia. Each ommatidium consists of 20 cells including eight photoreceptor cells, four cone cells and pigment cells. The normal compound eye of *Drosophila* is composed of approximately 750 ommatidia that are arranged in a regular hexagonal pattern. Since R3, R4, R1, R6, R7 and cone cells are recruited behind the morphogenetic furrow, where active proliferation has ceased[26], extra amounts of Ten-a in these cells should not cause defects in eye size. Instead, the presence of black ommatidia is consistent with cell death occurring later in eye development, once ommatidial cells have fully differentiated.

More severe defects in the eye were observed when overexpression of *ten-a* was induced using the *GMR*-Gal4 driver line. In the *GMR*-Gal4 line, the *GMR* enhancer drives expression of the Gal4 protein in cells of the developing eye including all cells within the morphogenetic furrow and posterior to it[27]. To overexpress *ten-a* posterior to the morphogenetic furrow, the GMR-Gal4 driver line was crossed to the UAS-*ten-a* lines. We found that the progeny from *GMR*-Gal4 x UAS-*ten-a 1x* showed dramatic defects in the compound eyes (Fig. 3E). Such defects included a disappearance of the hexagonal array of ommatidia, a large black patch in the eye, a lack of the eye color in the internal region of the eye surface and a slight

Table 2. The number of unhatched larvae.

	Number of counted larvae	Number of hatched larvae	Number of unhatched larvae	%
dsRNA	266	157	109	41
Buffer	248	225	23	9.7

reduction in size.

The disappearance of the hexagonal array and eye color indicated that the differentiation of cone cells and pigment cells was disturbed. The black patch in the eye indicated the occurrence of cell death. Moreover, larvae from *GMR*-Gal4 x UAS-*ten-a 2x* were very small and could not develop to the pupa stage. However, if the larvae were reared at 18 °C instead of 25 °C, they could survive and developed into adults with defects in the eyes. The defect was similar to those of the *GMR*-Gal4 x UAS-*ten-a 1x* cross (Fig. 3F). Since *GMR*-Gal4 induced *ten-a* overexpression in all cells of the morphogenetic furrow and posterior to it, it was likely that too much Ten-a product during this stage of eye development could disturb differentiation of ommatidial cells. However, eye defects do not affect viability of the fly[28]. Therefore, the death of larvae from the *GMR*-Gal4 x UAS-*ten-a 2x* cross might be due to Ten-a protein produced enormously. The excess protein might expand to other important cells or tissues and disturb some cell-cell interactions, leading to premature lethality.

Another driver line, *ey*-Gal4, was used to drive *ten-a* over-expression in the eye. In this driver, the promoter of the *eyeless* (*ey*) gene drives Gal4 expression in the embryonic eye primordium and, once the morphogenetic furrow starts sweeping across the disc, expression is restricted to all cells ahead of the furrow[29]. Using this driver, ectopic *ten-a* expression caused obvious defects in the compound eyes, which appeared small and rough. However, the hexagonal pattern was relatively normal. The eyes of the progeny from *ey*-Gal4 x UAS-*ten-a 2x* were smaller than those from *ey*-Gal4 x USA-*ten-a 1x* (Fig. 3H, I). It seems likely that an excess of Ten-a can interfere with cell proliferation resulting in the small eyes. Moreover, the more Ten-a protein is produced during this early stage of eye development, the smaller is the size of the eye.

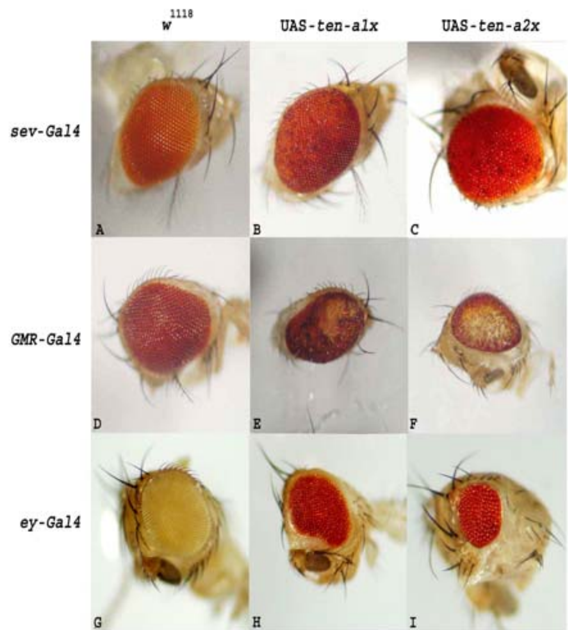

Fig 3. Defects in compound eyes caused by overexpression of *ten-a*. Overexpression of *ten-a* in the compound eyes induced by *sev*-Gal4 (A – C), by *GMR*-Gal4 (D – F) and by *ey*-Gal4 (G – I). See text for detail. (A, D, G) no defects in the compound eyes of the progeny from crosses between each Gal4 line and the w^{1118} line were observed. (B, E, H) The compound eyes of flies from a *sev*-Gal4 x UAS-*ten-a 1x* cross (B) a *GMR*-Gal4 x UAS-*ten-a 1x* cross (E) or a *ey*-Gal4 x UAS-*ten-a 1x* cross (H). (C, F, I) The compound eyes of flies from a cross between *sev*-Gal4 (C), GMR-Gal4 (F) or *ey*-Gal4 (I) and UAS-*ten-a 2x*. All flies were reared at 25°C except the flies carrying GMR-Gal4; UAS-*ten-a* 2x, that were reared at 18°C.

Discussion

ten-a is Required for Commissure Formation

We have shown that Ten-a is a transmembrane protein required for commissure formation. dsRNAi experiments suggested that Ten-a is required during early phases of commissure formation for axons to cross the midline through the proper commissure. Indeed, impairing the function of *ten-a* caused significant axon misrouting, leading to the fuzzy or fused appearance of commissures observed with mAb BP 102 staining. Many molecules regulating axon crossing of the midline have been reported and shown to be essential for commissure formation. Among such molecules, the best studied are the Netrins[30] and Slit[31], both expressed at the midline, as well as their respective receptors Frazzled[32] and the Robos[21]. However these receptors are expressed in both commissures, and mutations in the corresponding genes affect their formation to the same extent[33]. So far, only Derailed has been reported to be a molecule specifically required in the anterior commissure[34]. It has been shown that Derailed is a receptor for Wnt5, a member of the *Drosophila* Wnt family[35]. While Netrins and Wnt5 are diffusible molecules acting at long range, other axon guidance molecules are membrane-associated and function in a cell contact mediated fashion[36]. Presumably Ten-a belongs to this second category of axon guidance molecules, and we presume that it is necessary during the initial phases of commissure formation. Ten-a might label the posterior commissure, allowing axons to distinguish between the two

commissures as they form and to join the proper one. The commissural defects caused by dsRNAi confirmed such hypothesis since these defects could be explained by the lack of a molecule guiding axons through the correct commissure. Consequently axons crossed the midline in a disorganized fashion, giving rise to the fuzzy appearance of commissures observed with mAb BP102 staining.

ten-a is a Novel Gene Required for Eye Development

We used the Gal4/UAS system to identify the function of *ten-a* in the compound eye. The system has been used successfully to investigate the function of many genes [25,28,37,38]. With this system, a wild-type *ten-a* transgene was overexpressed in the eye by using three different Gal4 driver lines. These driver lines induced *ten-a* overexpression at different phases of eye development. We show that extra amounts of Ten-a protein can interfere with eye development during all phases. During the early phase, *ey*-Gal4 was used to overexpress *ten-a* anteriorly to the morphogenetic furrow.

Cells in this region did not differentiate but kept dividing to increase the cell number of the eye primordium. The small and rough eyes induced by overexpressing *ten-a* with *ey*-Gal4 indicated that Ten-a protein might have disturbed proliferation or might promote cell death, leading to a decrease in the eye size. To overexpress *ten-a* during the differentiation phase, we used *GMR*-Gal4 to induce *ten-a* overexpression posterior to the morphogenetic furrow. In this region, cells were differentiating to form ommatidia. Flies with extra amounts of Ten-a protein in this region exhibited eye defects such as no lens, no eye color and ommatidial death. These defects were the results of impairing the differentiation of ommatidial cellular components. *sev*-Gal4 induced *ten-a* overexpression in specific cells of the developing ommatidia. With this driver line, we found black ommatidia in the eye. These black ommatidia were degenerated ommatidia. This indicated that extra Ten-a amounts in specific cells of the developing ommatidia also promoted ommatidial death.

From our above findings, we would like to propose that *ten-a* is a novel gene required for normal eye development and that the Ten-a protein might function during several phases of eye development. Indeed, cell death was likely to occur with all three Gal4 lines used for this study although they induced Ten-a protein activity above normal threshold levels during different phases of eye development. During *Drosophila* eye development, apoptosis is a normal event to remove cells that have failed to adopt a proper fate within the eye tissue. Many genes involved in the apoptotic process have been identified, for example, *reaper, Dronc, DmPpt1, klumpfuss*. Overexpression of these genes induced small eyes and black ommatidia [25,28,38,39]. Therefore, we also suggest that the Ten-a protein might be another protein involved in the cell death mechanism of the eye. However the specific nature of such defects and the role played by Ten-a during the eye patterning events still need to be elucidated.

Acknowledgements

We thank Sol da Rocha for technical assistance, David Merritt for correcting the manuscript and the Bloomington Stock Center for fly stocks. This work was supported by grants from the Swedish "Vetenskapsrådet", the Swedish "Stiftelse för Strategisk Forskning" (Neurobiology Programme), the Segerfalk Foundation and Department of Genetics, Faculty of Science, Kasetsart University.

References

1. Baumgartner S and Chiquet-Ehrismann R (1993) *ten^a*, a Drosophila gene related to tenascin shows selective transcript localization. *Mech Dev* **40**, 165-76.
2. Wilson R, Ainscough R, Anderson K, Baynes C, Berks M, Bonfild J, Burton J, Connell M, Copsey T and Cooper J (1994) 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans*. *Nature* **368**, 32-8.
3. Minet AD, Rubin BP, Tucker RP, Baumgartner S and Chiquet-Ehrismann R (1999) Teneurin-1, a vertebrate homologue of the *Drosophila* pair-rule gene *ten-m*, is a neuronal protein with a novel type of heparin-binding domain. I. *Cell Sci* **112**, 2019-32.
4. Oohashi T, Zhou XH, Feng K, Richter B, Morgelin M, Perez MT, Su WD, Chiquet-Ehrismann R, Rauch U and Fassler R (1999) Mouse ten-m/Odz is a new family of dimeric teype II transmembrane proteins expressed in many tissues. *J Cell Biol* **145**, 563-77.
5. Otaki JM and Firestein S (1999) Neurestin: putative transmembrane molecule implicated in neuronal development. *Dev Biol* **212**, 165-81.
6. Minet AD and Chiquet-Ehrismann R 2000. Phylogenetic analysis of teneurin genes and comparison to the rearrangement hot spot elements of *E. coli*. *Gene* **257**, 87-97.
7. Baumgartner S, Martin D, Hagios C and Chiquet-Ehrismann R (1994) Ten-m, a *Drosophila* gene related to tenascin, is a new pair-rule gene. *EMBO J* **13**, 3728-40.
8. Chiquet-Ehrismann R, Matsuoka Y, Hofer U, Spring J, Bernasconi C and Chiquet M (1991) Tenascin variants: differential binding to fibronectin and distinct distribution in cell cultures and tissues. *Cell Regul* **11**, 927-37.
9. Levine A, Bashan-Ahrend A, Budai-Hadrian O, Gartenberg D, Menasherow S and Wides R. (1994) Odd Oz: a novel Drosophila pair-rule gene. *Cell* **77**, 587-98.
10. Fascetti N and Baumgartner S (2002) Expression of Drosophila *ten-a*, a dimeric receptor during embryonic development. *Mech Dev* **114**, 197-200.
11. Rubin GM and Spradling AC (1982) Genetic transformation of *Drosophila* with transposable elements vectors. *Science* **218**, 348-53.
12. Kennerdell RJ and Carthew WR (1998) Use of ds RNA-mediated genetics interference to demonstrate that *frizzled*

and *frizzled* 2 act in the wingless pathway. *Cell* **95**, 1017-26.
13. Patel NH (1994) Imaging neuronal subsets and other cell types in whole-mount *Drosophila* embryos and larvae using antibody probes. In: *Method in cell biology, Volume 44* (Edited by Glodstein LSB and Fryberg EA) pp 445-87. Academic Press, San Diego.
14. Brand AH and Perrimon N (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-9.
15. Fire A, Xu S, Montgomery MK., Kostas SA, Driver SE and Mello CC (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**, 806-11.
16. Ngo H, Tschudi C, Gull, K and Ullu E (1998) Double-stranded RNA induced mRNA degradation in *Trypanosoma brucei*. *Pro Natl Acad Sci USA* **95**, 14687-92
17. Wianny F and Zernicka-Goetz M (2000) Specific interference with gene function by double-stranded RNA in early mouse development. *Nat Cell Biol* **2**, 70-5.
18. Chuang CF and Meyerowitz EM (2000) Specific and heritable genetic interference by double-stranded RNA in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* **97**, 4985-90.
19. Hammond SM, Caudy AA and Hannon GJ (2001) Post-transcriptional gene silencing by double-stranded RNA. *Nature* **2**, 110-9.
20. Seeger M, Tear G, Ferres-Marco D and Goodman CS (1993) Mutations affecting growth cone guidance in *Drosophila*. Genes necessary for guidance toward or away from midline. *Neuron* **10**, 409-26.
21. Kidd K, Brose K, Mitchell KJ, Fetter RD, Tessier-Lavigne M, Goodman CS and Tear G (1998) Roundabout controls axon crossing of the CNS midline and defines a novel subfamily of the evolutionary conserved guidance receptors. *Cell* **92**, 205-15.
22. Van Vactor D, Sink H, Fambrough D, Tsoo R and Goodman CS (1993) Genes that control neuromuscular specificity in *Drosophila*. *Cell* **73**, 1137-53.
23. Bowtell DD, Kimmel BE, Simon MA, and Rubin GM. (1989) Regulation of the complex pattern of sevenless expression in the developing *Drosophila* eye. *Proc Natl Acad Sci USA* **86**, 6245-9.
24. Rorth P (1996) A modular misexpression screen in *Drosophila* detecting tissue-specific phenotypes. *Proc Natl Acad Sci USA* **93**,12418-22.
25. Korey CA and MacDonald ME (2003) An over-expression system for characterizing *PptI* function in Drosophila. *BMC Neuroscience* **4**, 30-9.
26. Wolff T and Ready DE (1993) Pattern formation in the *Drosophila* retina. In: *The Development of Drosophila melanogaster Vol. II* (Edited by Bate m and Martinez-arias A) pp. 1277-324. Cold Spring Habour Laboratory Press. New York.
27. Moses K and Rubin GM (1991) *glass* encodes a site-specific DNA-binding protein that is regulated in response to positional signals in the developing *Drosophila* eye. *Genes Dev* **5**, 583-93.
28. Hay BA, Maile R, and Rubin GM (1997) P element insertion-dependent gene activation in the *Drosophila* eye. *Proc Natl Acad Sci USA* **94**, 5195 – 200.
29. Hazelett DJ, Bourouis M, Walldorf U and Treisman JE (1998) *decapentaplegic* and *wingless* are regulated by *eye absent* and *eyegone* and interact to direct the pattern of retinal differentiation in the eye disc. *Development* **125**, 3741 – 51.
30. Mitchell KJ, Doyle JL, Serafini T, Kennedy TE, Tessier-Lavigne M, Goodman CS and Dickson BJ (1996) Genetic analysis of *netrin* genes in Drosophila: Netrins guide CNS commissural axons and peripheral motor axons. *Neuron* **17**, 203-15.
31. Kidd T, Bland KS and Goodman CS (1999) Slit is the midline repellent for the robo receptor in *Drosophila*. *Cell* **96**, 785-94.
32. Kolodziej PA, Timpe LC, Mitchell KJ, Fried SR, Goddman CS, Jan LY and Jan YN (1996) *frazzled* encodes a Drosophila member of the DCC immunoglobulin subfamily and is required for CNS and motor axon guidance. *Cell* **87**, 197-204.
33. Dickson BJ (2002) Molecular mechanisms of axon guidance. *Science* **298**, 1959-64.
34. Callahan CA, Muralldhar MG, Lundgren SE, Scully AL and Thomas JB (1995) Control of neuronal pathway selection by a *Drosophila* receptor protein-tyrosine kinase family member. *Nature* **376**, 171-4.
35. Yoshikawa S, McKinnon RD, Kokel M and Thomas JB (2003) Wnt-mediated axon guidance via *Drosophila* Derailed receptor. *Nature* **422**, 583-8.
36. Goodman CS, Bastiani MJ, Doe CQ, Lac S, helfand SL, Kuwada JY and Thomas JB (1984) Cell recognition during neuronal development. *Science* **225**, 1271-9.
37. Carrera P, Abrell S, Kerber B, Walldorf U, Preiss A, Hoch M and Jackle H (1998) A modifier sceen in the eye reveals control genes for Kruppel activity in the Drosophila embryo. *Proc Natl Acad Sci USA* **95**, 10779-84.
38. Rusconi JC, Fink JL and Cagan R. (2004) *Klumpfuss* regulate cell death in the *Drosophila* retina. *Mech Dev* **121**, 537-46.
39. Quinn LM, Dorstyn L, Mills K, Colussi PA, Chen P, Coombe M, Abrams J. Kumar S and Richardson H (2000) An essential role for the caspase Dronc in developmentally programmed cell death in *Drosophila*. *J Biol Chem* **275**, 40416-24.